Mycotoxins contamination of market maize and the potential of density sorting in reducing exposure in unregulated food systems in Kenya

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Abstract

Aflatoxins and fumonisins commonly contaminate key food staples in tropical countries, causing recurring acute and chronic public health problems. The present study was conducted to assess the potential of a simple device designed for density-based sorting of maize for its potential to reduce aflatoxins and fumonisins in diverse samples of naturally contaminated maize. A cross sectional survey was conducted, analysing market maize samples (n=204) from eight counties in Western Kenya. A quarter (25%) of the maize samples were contaminated with aflatoxin B₁ above the legal limit of 5 µg/kg and nearly half (48%) were contaminated with fumonisins at levels above the legal limit of 2 µg/g. Analysis of additional samples (n=24) from Meru County showed that contamination of maize with the two toxins was also common in Eastern Kenya. A simple density sorter was used to separate grain samples into heavy and light fractions. With an out-sort rate of 31%, density sorting was effective in separating maize by bulk density and 100-kernel weight ($P < 0.001$). Bulk density was negatively correlated with aflatoxins in unsorted ($r = -0.20$, $P < 0.01$) and heavy fractions ($r = -0.32$, $P < 0.01$). Density sorting was effective at reducing fumonisins; for maize samples with > 1 µg/g, the heavy (accepted) fraction had 66% lower fumonisins than the unsorted maize. After density sorting, the light and heavy fractions fumonisin levels differed by an average of 8.38 µg/g ($P < 0.001$). However, sorting was not effective at significantly reducing aflatoxin levels in maize that was highly contaminated. A simple density sorting equipment that could be adopted by local small-scale millers has potential to reduce fumonisins in maize. Additional and complementary sorting technologies, such as size screening and spectral sorting might improve the effectiveness of reducing aflatoxins in maize.

Keywords: aflatoxins, fumonisins, food safety, density sorting, maize

1. Introduction

Mycotoxins are toxic fungal metabolites that contaminate up to 60-80% of human food staples globally, with a quarter of foodstuffs contaminated above legal levels (Eskola *et al.*, 2019). Maize is particularly vulnerable to colonisation by the fungi that produce aflatoxins (AF) and fumonisins (FUM) (Mutiga *et al.*, 2015). Groundnuts are also highly susceptible to AF producing fungi. In East Africa, maize

and groundnuts are commonly consumed and contribute to frequent dietary mycotoxin exposure. The hot and humid climatic conditions favour the growth and multiplication of toxigenic moulds, such as *Aspergillus flavus* and *Aspergillus parasiticus*, which produce AF, and *Fusarium verticillioides*, which produces FUM. Further accumulation of mycotoxins occurs due to inadequate drying of the crop and subsequent poor storage (Chulze, 2010).

In East Africa, where such conditions and practices are common, high levels of AF and FUM have been reported in several studies. For example, outbreaks of acute aflatoxicosis have been reported frequently in Kenya (Daniel *et al.*, 2011) and occasionally in Tanzania (Kamala *et al.*, 2018; Kimanya, 2015). In 2007, the first year since 2004 when no aflatoxicosis outbreaks were reported, widespread exposure to AF was found across all provinces in Kenya, including those with no evidence of previous exposure and history of acute aflatoxicosis (Yard *et al.*, 2013). Notably, Kenya's Eastern Province is prone to high levels of AF exposure (Daniel *et al.*, 2011; Mutiga *et al.*, 2014; Yard *et al.*, 2013). This is attributed to the genetically unique and extremely toxigenic S strain morphotype of *A. flavus* (Probst *et al.*, 2012).

A cross-sectional survey conducted in 2009, with maize samples from 985 patrons of local hammer mills, found 15% of maize flour samples with a higher aflatoxin level than 10 µg/kg, across three administrative regions in Western Kenya (Mutiga *et al.*, 2015). A similar survey conducted in 2010, in which milled maize flour samples were gathered from 1,500 mill patrons from 10 administrative districts in Eastern Kenya were analysed, found that 39% of the samples were above the legal limit (10 µg/kg) for AF, and between 22 and 60% of samples above 10 µg/kg across the 10 districts (Mutiga *et al.*, 2014).

Although AF can accumulate after harvest, a recent study showed significant levels of pre-harvest AF contamination of maize in eastern and south-western Kenya (Mahuku *et al.*, 2019). Of the samples with detectable AF, 56% (153/274) from eastern and 44% (102/233) from south-western Kenya exceeded 5 µg/kg for aflatoxin B₁ (AFB₁) in human food (the maximum allowable limit; 10 µg/kg is the legal maximum for total AF). While considerable effort has been made to document aflatoxin contamination in African food systems, less attention has been invested in understanding the extent of FUM contamination. In fact, there are no legal regulatory limits or means to implement food safety protection with regard to FUM in most Sub-Saharan countries, especially in low-income contexts characterised by poor agronomic practices, sub-optimal pre- and post-harvest management of maize, and a highly unregulated value chains.

AF and FUM exposure have adverse health effects. AF causes liver cancer in humans and is classified as a group 1 carcinogen (IARC, 2012). FUM exposure is considered an environmental risk factor for neural tube defects (Gelineau-van Waes *et al.*, 2009). FUM exposure has been associated with oesophageal cancer in several countries, including Kenya (Bolger *et al.*, 2001; Kigen *et al.*, 2017; Shephard *et al.*, 2009). While there is evidence of carcinogenicity of FUM in experimental animals, the evidence linking FUM exposure with cancer in humans is inadequate, so FUM is categorised as a group 2B carcinogen (IARC, 2002).

Dietary AF exposure is associated with linear growth faltering in infants and young children (IYC). A cross-sectional study in Benin and Togo showed a clear dose-response relationship between aflatoxin-albumin (AF-alb) concentration, as well as for Z-scores for height-for-age and weight-for-age (Gong *et al.*, 2002). AF-alb is a blood biomarker, which shows chronic exposure to dietary AF over the last 2-3 months. AF-alb was also associated with reduced height of children in a longitudinal study in Benin (Gong *et al.*, 2004), and was especially high among fully weaned children. Maternal exposure to AF during pregnancy was associated with lower height and weight in the first year of life in Gambian infants (Turner *et al.*, 2007). FUM exposure has been associated with poor linear growth among young rural Tanzanian children (Kimanya *et al.*, 2010; Shirima *et al.*, 2015).

AF and FUM are known to co-occur in maize (Hove *et al.*, 2016; Kimanya *et al.*, 2008), which forms a large component of complementary food for infants as young as 3 months (Magoha *et al.*, 2016). Considered together, the observational evidence strongly suggests AF and FUM exposure could play a significant role in linear growth faltering, although the causal evidence is not definitive. Given the widespread exposure to AF and FUM, and their potential role in contributing to linear growth faltering and adverse public health effects in low and middle income contexts, it is important that effective, cost-effective and scalable interventions are developed to reduce the burden of exposure to these toxins from common staples, especially in contexts where (a) maize and groundnuts are commonly consumed, (b) food insecurity is high, (c) dietary diversity is low and (d) inadequate infant and young child feeding practices are common, including early introduction of complementary feeding.

In contaminated maize samples, AF and FUM are often unevenly distributed. Most of the toxin in contaminated samples is carried by a small proportion of the grains (Grenier *et al.*, 2014). Although maize kernels that have been colonised by toxigenic fungi may or may not be observably different from those that are non-toxic (Mutiga *et al.*, 2014) kernels with high levels of AF and/or FUM may have slightly different physical properties compared to clean kernels, including lower density and size (Aoun *et al.*, 2020). On this basis, we hypothesise that density sorting can be used to reduce toxins (Grenier *et al.*, 2014; Huff and Hagler, 1985).

This study provides further evidence of mycotoxin contamination of maize sampled from several counties in the Eastern, Western, Nyanza and Rift Valley provinces of Kenya. The findings indicate widespread FUM contamination in maize from markets in Kenya, extending our previous observations (Mutiga *et al.*, 2015). The study

provides evidence on the potential of density sorting using to reduce FUM in maize (Nelson, 2016).

2. Materials and methods

Maize samples

In November 2017, a diverse set of maize samples ($n=204$; varying in terms of variety/seed type and area of origin) were collected from 57 local markets in 8 counties in Nyanza, Western and Rift Valley Provinces of Kenya (Figure 1). About 5 to 10 maize samples were taken from each market based on accessibility. Approximately 2 kg of maize was randomly drawn from each trader's maize and carefully sealed in plastic/polythene zip locks bags with minimum air trapped. The samples were transported to the Biosciences East and Central Africa Hub at the International Livestock Research Institute (BecA-ILRI) in Nairobi and stored at 4 °C prior to analysis. To further diversify the pool of samples for sorting experiments, 24 additional maize samples from Meru County in Eastern Province of Kenya were requested

from and provided by colleagues within the MAICE trial ('Mitigating aflatoxin exposure to improve child growth') conducted in eastern Kenya.

As much as sample collection was done in 8 counties, we checked with each trader to find out the origin of the maize. Overall, the maize collected was sourced from 13 counties. Further, not all the maize samples collected met the criteria for the sorting experiment described below. Only maize from 7 counties of origin met those criteria.

Sample preparation and mycotoxin analyses

A sub-sample of 400 g from each sample was drawn from each thoroughly mixed bulk sample. The 400 g were milled to approximately 0.5 mm particle size using a Romer Series II sub-sampling mill (Romer Labs, Getzersdorf, Austria).

AFB₁ was extracted from 5 g with 25 ml of 70% methanol in an orbital shaker (New Brunswick Scientific, Edison, NJ, USA) at 350 rpm for 10 min. AFB₁ was analysed using a

Figure 1. Map showing 57 markets where maize was collected within eight counties and elevation.

commercially available ELISA kit from Helica Biosystems Inc. (Fullerton, CA, USA) following the manufacturer's instructions. Samples with AFB₁ below 1 µg/kg were considered non-detectable, and those beyond 20 µg/kg were diluted with the extraction solvent and re-tested.

To extract FUM, 5 g of maize flour was mixed with 25 ml of 90% methanol to achieve a 5× dilution of the sample, and shaken in an orbital shaker (New Brunswick Scientific) at 350 rpm for 10 min. Further dilution with distilled water was done to achieve dilution of 40× for all the samples. FUM was analysed using a commercial ELISA kit following the manufacturer's protocol (Helica Biosystems Inc.). The solid-phase direct competitive kit (Helica Biosystems Inc.) is a 96-well microplate coated with an antibody optimised to cross-react with three FUM subtypes (B₁, B₂ and B₃). The lower and upper limits of detection were 1 and 6 µg/g, respectively. Samples with greater than 6 µg/g were diluted further and re-tested until the sample was within the assay's valid range.

Optical density (OD) readings of the reactions for each toxin were obtained using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) with an absorbance filter of 450 nm. The ODs of the samples were compared to ODs of the standards included in each experiment and the results were interpreted. For quality control purposes an in-house analytical method for performance characteristics developed for assessing the accuracy, precision and linearity for each ELISA plate was adopted (Nishimwe *et al.*, 2017).

Density sorting of the selected maize samples

To determine the effectiveness of density sorting on reducing AF and FUM, we took sub-samples of 300 g each from the original batches as collected from the markets. These sub-samples were subjected to density without further drying. This was identified as the 'WET' experiment. A similar set of sub-samples were dried overnight in oven at 60 °C before density sorting. This was identified as the 'DRY' experiment.

Samples from the November 2017 survey that exceeded 50 µg/kg for AFB₁ and 2 µg/g for FUM were selected for density sorting. After thorough mixing of the initial 2 kg sample, sub-samples of 300 g each were drawn. In total 4 sub-samples were taken from each of the 20 maize samples (that is, two sub-samples for each experiment) to add up to 80 sub-samples. These were further sorted into 160 fractions. Each sub-sample was separated into a light and heavy fraction using a DropSort device (Nelson, 2016). The light fraction was weighed after every sorting round until 25-35% of the 300 g was separated.

An additional 24 samples from Meru County were sorted into 48 fractions. Since AF and FUM distribution in maize is heterogeneous, all sub-samples were treated as independent samples of maize in data analysis.

Multi-mycotoxin analysis in maize samples

Multiple mycotoxins were analysed using an LC-MS/MS method adapted from other sources (Sameni *et al.*, 2014; Sulyok *et al.*, 2006), modified and validated for maize by BECA/ILRI Hub, Nairobi, Kenya.

Five grams (5.0±0.1 g) of each sample were accurately weighed into a 50 ml falcon tubes. Extraction was carried out with 20 ml extraction solution (acetonitrile/water/formic acid, 79:20:1, v/v/v) on a mechanical orbital shaker (New Brunswick Scientific) for 90 min at 220 rpm. After extraction, 200 µl of the sample extract and 800 µl of the mobile phase (formic acid, ammonium acetate and water) were pipetted into a 2 ml Eppendorf tube and vortexed. The diluted extract was filtered through 0.2 µm nylon microfilters into an amber coloured UPLC vial with the help of a 2 ml syringe, vial capped and stored at -80 °C prior to LC-MS/MS analysis.

Mycotoxins were identified and quantified using a Shimadzu 8050 LC-MS/MS system equipped with an ion electrospray ionization source operating in both positive and negative ionization modes (Shimadzu Corp., Kyoto, Japan). Data acquisition was carried out in the multiple reaction-monitoring (MRM) mode. The MRM conditions were optimised for the different analytes through flow injection analysis of the standards. Parameters optimised for each analyte were Cone voltages (CV) and collision energies (CE) for the associated product ions.

Performance characteristics of the optimised method were established by a validation procedure with spiked maize samples by evaluating linearity, repeatability, limits of detection (LOD) and limits of quantitation (LOQ), using both standards in solvent and those in matrix. Linearity was evaluated using the determination coefficients (R²). The acceptable value of the curve was >0.99.

Statistics

AF and FUM data were highly skewed and were natural log transformed prior to statistical analysis. All data analysis was done using the publicly available R software (RStudio 1.2.50).

3. Results

Aflatoxins and fumonisins by county

A quarter (25%) and nearly half (48%) of the maize samples from 8 counties in Kenya were contaminated with AFB₁ and FUM at levels above the legal limit of 5 µg/kg and 2 µg/g, respectively (Table 1). 80 and 85% of the samples had detectable AFB₁ and FUM, respectively, suggesting more widespread chronic exposure to the two toxins. 17% of the samples had both toxins greater than the legal limits. There was great variability in contamination of the two toxins across the counties (Figure 2).

Maize from Meru County had high levels of total AF with 92% of the samples exceeding the legal limit of 10 µg/kg (median: 2,480 µg/kg, range: 1.2-16,414.0 µg/kg). 58% of these samples had levels of FUM above 2 µg/g (median: 2.7, range: 0.1-15.3 µg/g).

Effectiveness of density sorting on grain properties

At an average 31% out-sort rate based on mass, sorting was effective in separating maize by bulk density by a mean difference of 0.05 g/ml between the heavy and light fractions (Table 2). Similarly, density sorting was effective in sorting maize by kernel weight, with a mean difference of 6.16 g per 100 kernels between light and heavy fractions ($P < 0.05$; Table 2). Bulk density and 100-kernel weight had a clear positive linear relationship as expected (Figure 3).

Effectiveness of density sorting on reducing aflatoxin levels

Density sorting was not effective at reducing AF concentration in the heavy or retained fraction ($P > 0.05$; Table 3, Figure 4A and 4B). The mean difference in AF between bulk (unsorted) sample and the heavy fraction was negative as opposed to the hypothesised positive value

Table 1. Occurrence and co-occurrence of aflatoxin B₁ (AFB₁) and fumonisin (FUM) in maize samples from Kenya (n=204).¹

Occurrence (n, %)				Co-occurrence		
AFB ₁		FUM		AFB ₁ (µg/kg)	FUM (µg/g)	
ND	40 (20%)	ND	30 (15%)		≤2	>2
≤5 (µg/kg)	113 (55%)	≤2 (µg/g)	75 (37%)	≤5	50 (25%)	43 (21%)
>5 (µg/kg)	51 (25%)	>2 (µg/g)	99 (48%)	>5	12 (6%)	35 (17%)

¹ The maize samples were analysed using ELISA. ND = not detected.

Figure 2. Variation of (A) aflatoxin B₁ (µg/kg) and (B) total fumonisin (µg/g) in maize (n=204) from 13 counties of origin. The maize samples were analysed using ELISA. The dotted line shows the legal limit in Kenya of aflatoxin B₁ (5 µg/kg) and fumonisin in Kenya (2 µg/g) respectively.

Table 2. Maize grain physical characteristics (n=49).¹

Fraction	Sort mass (%) Mean (SD)	Bulk density (g/ml) Mean (SD)	100 kernel weight (g) Mean (SD)
Heavy	68.2 (4.2)	0.74 (0.03)	32.9 (4.6)
Light	31.3 (4.4)	0.68 (0.03)	26.7 (3.0)
Combined	49.7 (19.1)	0.71 (0.04)	29.8 (5.0)
Difference (heavy-light)	36.97	0.05	6.16
Pr (T > t)	0.000	0.000	0.000

¹ SD = standard deviation.

Figure 3. Scatter plot showing bulk density by 100-kernel weight for the heavy and light fraction, with linear model best-fit line and standard error shading. Light fractions are shown in white and heavy fractions in black.

Table 3. Density sorting for aflatoxins (unsorted samples with >20 µg/kg aflatoxins).¹

Variable	Total aflatoxins (µg/kg)		Log total aflatoxins	
	n	Mean (SD)	n	Mean (SD)
Heavy fraction	70	1,886 (4,383)	58	5.06 (2.58)
Light fraction	71	1,574 (3,896)	62	4.90 (2.44)
Combined	141	1,729 (4,133)	120	4.97 (2.50)
Difference		312		0.16
Pr (T > t)		0.66		0.72

¹ SD = standard deviation.

Figure 4. (A) Differences in total aflatoxins (µg/kg) by maize fractions ($P=0.72$). The data was first transformed using natural logarithm function. (B) Variation in total aflatoxins (µg/kg) between maize fractions by county of origin (light fractions are shown in white and heavy fraction in gray). Transformation of the data using the formula Logarithm with base 10 of (AF in µg/kg +1) was done to fit all values within the y-axis scale and avoid infinite values. (C) Log of differences between light and heavy aflatoxins (µg/kg) by DRY and WET experiments ($P>0.05$). The data was first transformed using natural logarithm function.

if density sorting was effective (Table 4). Oven drying the maize samples to between 11.5-13% did not affect or improve the effectiveness of sorting for total AF (Figure 4C). However, oven drying increased variability between the light and heavy AF levels within samples.

However, bulk density was significantly and negatively associated with AF levels in unsorted maize ($r=-0.20$,

$P<0.01$) and heavy fraction ($r=-0.32$, $P<0.01$) (Table 5 and Figure 5), such that an increase in bulk density predicted lower AF. An insignificant negative trend was observed for bulk density and AF levels in the light fraction. A 100-kernel weight was not significantly associated with AF levels in either fraction (Figure 5).

Table 4. Difference between maize fractions total aflatoxins (AF in µg/kg).

Variable	n	Mean	Min	Max	Anticipated trend ¹
Light-heavy (ALL)	63	206 (1,808)	-3,766	8,987	Positive
% difference light-heavy	55	-40.3 (310.4)	-2,046	100	Positive
Light-heavy (positive values)	39	670 (2,000)	0.89	8,987	
Light-heavy (negative values)	20	-657 (1,196)	-3,766	-1.11	
Unsorted (Bulk)-heavy	49	-142 (2,593)	-8,824	9,806	Positive
% difference bulk-heavy	49	-7.1 (126)	-649	100	Positive
Weighted-heavy	38	83 (829)	-1,299	3,533	Positive
% difference weighted-heavy	34	24 (41)	-43	100	Positive

¹ Anticipated: based on our hypothesis that sorting is effective in reducing AF.

Table 5. Correlation analysis of aflatoxin (AF) and fumonisin (FUM) levels.¹

	Bulk density (g/ml)	Heavy fraction FUM (µg/g)	Unsorted FUM (µg/g)
100 Kernel weight (g)	0.46 ***	0.38 **	0.20 *
Unsorted AF (µg/kg)	-0.20 *		
Log unsorted AF (µg/kg)	-0.30 **		
Heavy fraction AF (µg/kg)	-0.32 *		
Heavy fraction log AF (µg/kg)	-0.43 **		
Light fraction AF (µg/kg)	-0.09 ns		
Light fraction log AF (µg/kg)	-0.26 ns		
Light fraction log FUM (µg/g)	-0.16 ns		
Heavy fraction log FUM (µg/g)	-0.01 ns		
Log unsorted FUM (µg/g)	-0.11 ns		
Unsorted FUM (µg/g)	-0.07 ns	0.45 ***	

¹ Significance: *** $P=0.000$, ** $P=0.01$, * $P=0.05$, ns = not significant.

Figure 5. (A) Scatter plot for bulk density by aflatoxins (µg/kg) in maize fractions. Light fraction: $r=-0.26$, $P>0.05$ (not significant). Heavy fraction: $r=-0.43$, $P<0.01$. (B) Scatter plot for 100 kernel weight by aflatoxins (µg/kg) in fractions. Light fraction: $r=-0.10$, $P>0.05$ (not significant). Heavy fraction: $r=0.08$, $P>0.05$ (not significant). The data was first transformed using natural logarithm function. Light fractions are shown in white and heavy fractions in black.

Effectiveness of density sorting in reducing fumonisin levels

At an out-sort rate of 31% mass, density sorting was effective in separating maize by bulk density with a difference of 0.054 g/ml between the heavy and light fractions ($P<0.05$). Similarly, density sorting resulted in a mean difference of 5.9 g in 100-kernel weight between the heavy and light fractions ($P<0.05$; Table 6). Density sorting was also effective in reducing FUM levels by an average of 8.38 µg/g difference

between the light and heavy fractions ($P<0.05$) (Table 6 and Figure 6A). However, it is clear that the density sorter was not entirely effective at reducing FUM to levels below the legal limit of 2 µg/g in most of the samples (Figure 6B). The average mean of FUM in heavy fractions was 2.32 µg/g (Table 6). A reduction of 65.5% in FUM from unsorted (bulk) maize to heavy fraction was observed for unsorted samples with FUM at >1 µg/g (Table 7). This changed to 66.5, 71.0 and 70.4% for unsorted samples with FUM at >2 µg/g ($n=56$), >4 µg/g ($n=48$) and >6 µg/g ($n=39$),

Table 6. Grain characteristics (n=53) and fumonisin (FUM) level in maize fractions.¹

Fraction	Sort mass (%)	Bulk density (g/ml)	100 kernel weight (g)	FUM (µg/g)		Log FUM	
	Mean (SD)	Mean (SD)	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Heavy	68.6 (4.2)	0.74 (0.03)	32.9 (3.8)	86	2.32 (2.82)	84	0.02 (1.59)
Light	30.8 (4.4)	0.69 (0.03)	27.0 (3.2)	85	10.70 (9.83)	84	1.53 (1.78)
Combined	49.7 (19.5)	0.71 (0.04)	30.0 (4.6)	171	6.49 (8.33)	168	0.77 (1.84)
Difference	37.8	0.054	5.9		-8.38		-1.51
Pr (T > t)	0.000	0.000	0.000		0.000		0.000

¹ SD = standard deviation.

Figure 6. (A) Differences in fumonisins (µg/g) levels between heavy and light maize fractions ($P=2.1e-07$). The data was first transformed using the natural logarithm function. (B) Variation in fumonisins (µg/g) between maize fractions by county of origin (light fractions are shown in white and heavy fractions in gray). The dotted line shows the legal limit of fumonisins in Kenya (2 µg/g). (C) Log of differences in fumonisins (µg/g) between light and heavy maize fractions by DRY and WET experiments ($P>0.05$). The data was transformed using natural logarithm function.

respectively. Oven drying the maize samples to between 11.5–13% did not affect or improve the effectiveness of sorting for FUM (Figure 6C).

Bulk density seems to be negatively correlated with FUM levels in the light fraction (Figure 7) but not statistically significant (Table 7). Similarly, 100-kernel weight seems to be negatively correlated with FUM in the light fraction (Figure 7), but not statistically significant ($r=-0.14$, $P>0.05$). 100-kernel weight was positively correlated with both FUM in unsorted maize and in the heavy fraction ($P<0.05$; Table

7). This observation was unexpected. It is likely that other drivers of kernel weight, such as genetic and nutrition, override the effect of fungus infestation.

About a quarter (24%) of the heavy fractions had levels of AF and FUM within the legal limits compared to none for the light fractions. Co-occurrence of the two toxins in the fractions was high and more so for the light fraction. More than a third (39%) of the light fractions had more than 10 µg/kg and 2 µg/g for total AF and FUM, respectively, compared to 17% of the heavy fractions (Table 8).

Table 7. Difference between maize fractions for fumonisins (µg/g).¹

Variable	n	Mean (SD)	Min	Max	Anticipated trend ²
Light-heavy	79	8.61 (8.99)	-2.50	32.50	positive
% difference light-heavy	73	72.03 (35.24)	-135.6	100.00	positive
Weighted mean-heavy	44	2.84 (2.99)	-0.63	9.99	positive
% difference weighted mean-heavy	44	46.32 (38.10)	-49.94	98.63	positive
Unsorted (bulk)-heavy	72	5.03 (4.70)	-3.56	19.76	positive
% difference bulk-heavy	72	65.50 (39.05)	-65.56	100.00	positive

¹ SD = standard deviation.
² In all comparisons shown, all expectations based on the hypotheses with regard to density and FUM levels were met, unlike the case with AF (Table 4).

Figure 7. (A) Scatter plot for bulk density by fumonisins (µg/g) in maize fractions. Light fraction: $r=-0.16$, $P>0.05$ (not significant). Heavy fraction: $r=-0.01$, $P>0.05$ (not significant). (B) Scatter plot for 100 kernel weight by fumonisins (µg/g) in fractions. Light fraction: $r=-0.14$, $P>0.05$ (not significant). Heavy fraction: $r=0.16$, $P>0.05$ (not significant). The data was first transformed using natural logarithm function. Light fractions are shown in white and heavy fractions in black.

Table 8. Co-occurrence of aflatoxin (AF) and fumonisin (FUM) in both maize fractions.

Total AF (µg/kg)	Light fraction Total FUM (µg/g)		Heavy fraction Total FUM (µg/g)	
	0-2 (µg/g)	>2 (µg/g)	0-2 (µg/g)	>2 (µg/g)
0-10 (µg/kg)	0 (0%)	32 (35%)	22 (24%)	19 (21%)
>10 (µg/kg)	24 (26%)	35 (39%)	35 (38%)	16 (17%)

4. Discussion

This cross-sectional survey of market maize from eight counties in Nyanza, Western and Rift Valley regions showed significant contamination of AFB₁, with 25% of samples showing AFB₁ at levels greater than the legal limit. In a prior study conducted in 2009, 15% of maize samples collected from the same regions had more than 10 µg/kg for total AF (Mutiga *et al.*, 2015). The highly contaminated samples acquired from Kenya's Eastern Province demonstrate the striking nature of the highly toxigenic S-strains of the *Aspergillus flavus* in Eastern Kenya, as seen in earlier studies (Probst *et al.*, 2012).

While the extent of AF contamination is notable, FUM contamination was higher and more widespread than AF across most of the counties in Kenya's Western Province. Widespread contamination of maize with FUM was also reported in a cross-sectional study of maize samples from the same region (Mutiga *et al.*, 2015). 50% of the maize samples had FUM levels greater than legal limit then (1 µg/g), similarly to the 48% (over the current regulatory limit of 2 µg/g) reported in this study. There was also significant co-occurrence of AF and FUM, with 17% of the samples above the legal limit for both toxins.

The extent of mycotoxin contamination can vary greatly from ear to ear and kernel to kernel (Lee *et al.*, 1980; Turner *et al.*, 2013). Consequently, mycotoxin contamination between and within batches of grain is highly uneven (Kabak *et al.*, 2006; Nelson, 2016). AF contaminated kernels tend to be less dense than AF-free kernels (Grenier *et al.*, 2014; Lee *et al.*, 1980). Since only a fraction of the kernels account for high AF levels in a given batch, there is potential for reduction of toxin levels by density separation of the highly toxic kernels. The same hypothesis could be extended to separating kernels contaminated with high levels of FUM. We were able to reduce FUM levels by density sorting using a simple device that was designed to be adaptable to local hammer mills. In contrast, we were unable to significantly reduce AF levels by density sorting. The co-occurrence of AF and FUM in the light and heavy fractions was inconsistent suggesting that the two were uncorrelated. However, there was a 24% increase in number of fractions within the legal limit between light and heavy fraction, and a 22% reduction in number of fractions above the legal limits, for both toxins.

It is likely that colonisation and metabolism of the maize kernels by *A. flavus* differ quite significantly from *F. verticilloides*, such that the effect on density of kernels differs substantially. *A. flavus* tends to colonise the maize ear through the silk channel, such that kernel infection is cryptic (internal), compared to *F. verticilloides* which tends to colonise the outer layers of the maize kernel (Shu *et al.*, 2017). *F. verticilloides* produces more toxin compared to

Aspergillus species, as reflected in the units of measure for FUM and AF (µg/g versus µg/kg). Therefore *F. verticilloides* is likely to utilise more and softer (starchy) grain matter, which translates to greater impact on kernel density.

Density sorting reduced FUM in heterogeneous maize in Kenya by 66% with removal of 31% of the light fraction. However, such losses are high for low-income, small-holder farmers. The mean FUM level in the heavy (accepted) fraction was close to the legal limit (2 µg/g). Further calibration of the device and size separation of maize kernels can help optimise sorting and reduce variability due to mixed varieties. Differences in kernel sizes and shapes contributed to differences in drag effect during density sorting using the DropSort device, which creates suction to draw lighter kernels towards one end of the device.

In several studies, hand (visual) sorting was effective in reducing FUM (Kimanya *et al.*, 2009; Matumba *et al.*, 2015; Mutiga *et al.*, 2014; Van der Westhuizen *et al.*, 2010). Mutiga *et al.* (2014), for example, reported 65% reduction in FUM levels between unsorted and visually sorted maize, from above to below legal limit of 1 µg/g. However, visual sorting has shown mixed results as far as reduction of AF is concerned. It was effective in reducing AFB₁ in maize by about 94% in Malawi (Matumba *et al.*, 2015), but not in Kenya (Mutiga *et al.*, 2014). While visual sorting can be effective in reducing FUM, it is laborious and inefficient, especially where large quantities of maize are involved.

This study demonstrates that density separation is ineffective in reducing AF. This is likely due to two reasons. Firstly, the maize samples were highly contaminated, such that kernels with high AF ended up in both fractions. Almost half (48%) and 30% of the unsorted samples had total AF higher than 100 and 1,000 µg/kg, respectively. Most of the samples with AF greater than 1,000 µg/kg were from Meru county in Eastern Province, an area known for the high toxigenic S strains of *A. flavus* (Probst *et al.*, 2012). Secondly, the differential effect on density driven by other factors like mixed seed or varieties, water stress and nutrition, masked any difference in density due to *A. flavus* colonisation. Future studies should consider size screening before density sorting to reduce variation in effectiveness of sorting driven by the aforementioned factors. Such studies should use less contaminated maize to establish if there is a threshold where density sorting can work.

Complementary approaches, such as spectral sorting, can further reduce AF and FUM in the accepted maize fraction. A mean of 83% reduction in AF and FUM was achieved using a low cost multi-spectral sorter with up to 25% of sample mass rejected for Kenyan maize that tested positive for the toxin (Stasiewicz *et al.*, 2017). Other strategies known to prevent post-harvest AF accumulation include hermetic storage using Purdue improved crop

storage (PICS) bags for maize below 14% moisture content (Ng'ang'a *et al.*, 2016).

Bulk density was negatively correlated with AF levels in both unsorted maize and the heavy fraction. Similarly, bulk density was negatively correlated with AF levels in Eastern Kenya maize (Mutiga *et al.*, 2017). Also known as test weight, bulk density is measured as the mass of a sample for a given volume of grain. Bulk density of a grain sample is an indicator of crop health (Mutiga *et al.*, 2017) and a consequence of the sample's genetic composition and environmental factors that have influenced it before, during and after harvest. Colonisation by mycotoxigenic fungi is just one of the environmental factors that can influence grain density. High density is likely to reduce the ease with which fungi can colonise a sample, which likely contributes to the negative correlation between bulk density and toxin levels. Bulk density can therefore be used as proxy for healthy or clean maize. Such a proxy can be adopted as a criterion in procuring AF safe maize in unregulated food systems in low and middle-income contexts. We recommend further research on the specificity and sensitivity of such a proxy in accepting or rejecting quality and safe maize.

5. Conclusions

While AF has received much attention in terms of research, regulation, policy and funding, it still remains an intractable and significant public health problem. Similar to hand (visual) sorting, our study shows that density sorting cannot reduce AF levels in a highly contaminated sample. It is therefore pertinent that integrated management of the AF problem in tropical countries like Kenya focuses on a whole value chain approach such as improving seeds, good agricultural practices, post-harvest management (e.g. the use of hermetic storage solutions), as opposed to relying on end of the value chain solutions like sorting.

Our study reinforces prior observations suggesting that FUM contamination is widespread in Kenya. Taken together with the available evidence that FUM is a health hazard, our findings imply that the extent of contamination pose a significant risk to human health. While there is increasing awareness of the dangers associated with AF in the African food system, FUM has received much less attention with regard to research, mitigation, regulation, policy and funding.

Density sorting can significantly reduce FUM contamination in maize. This simple technology has great potential to be adapted at local hammers mills to minimise FUM exposure in weakly regulated food systems. Mechanical sorting is more efficient and less labour intensive compare to hand/visual sorting. However, it is imperative that incentives be created along the maize value chain to ensure the high out-

sort rate is carefully managed in an economically gainful way and to eliminate the threat of out-sorts finding their way back into the local food system. Future sorting efforts should focus on using a combination of complementary technologies such as size screening, density and spectral sorting to improve on efficacy.

Conflict of interest

The authors declare no conflict of interest.

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