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Multi-spectral kernel sorting to reduce aflatoxins and fumonisins in Kenyan maize

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ABSTRACT

Maize, a staple food in many African countries including Kenya, is often contaminated by toxic and carcinogenic fungal secondary metabolites such as aflatoxins and fumonisins. This study evaluated the potential use of a low-cost, multi-spectral sorter in identification and removal of aflatoxin- and fumonisin-contaminated single kernels from a bulk of mature maize kernels. The machine was calibrated by building a mathematical model relating reflectance at nine distinct wavelengths (470–1550 nm) to mycotoxin levels of single kernels collected from small-scale maize traders in open-air markets and from inoculated maize field trials in Eastern Kenya. Due to the expected skewed distribution of mycotoxin contamination, visual assessment of putative risk factors such as discoloration, moldiness, breakage, and fluorescence under ultra-violet light (365 nm), was used to enrich for mycotoxin-positive kernels used for calibration. Discriminant analysis calibration using both infrared and visible spectra achieved 77% sensitivity and 83% specificity to identify kernels with aflatoxin >10 ng g^{-1} and fumonisin >1000 ng g^{-1}, respectively (measured by ELISA or UHPLC). In subsequent sorting of 46 market maize samples previously tested for mycotoxins, 0–25% of sample mass was rejected from samples that previously tested toxin-positive and 0–1% was rejected for previously toxin-negative samples. In most cases where mycotoxins were detected in sorted maize streams, accepted maize had lower mycotoxin levels than the rejected maize (21/25 accepted maize streams had lower aflatoxin than rejected streams, 25/27 accepted maize streams had lower fumonisin than rejected streams). Reduction was statistically significant ($p < 0.001$), achieving an 83% mean reduction in each toxin. With further development, this technology could be used to sort maize at local hammer mills to reduce human mycotoxin exposure in Kenya, and elsewhere in the world, while at once reducing food loss, and improving food safety and nutritional status.

1. Introduction

Mycotoxins are toxic secondary metabolites of fungi that contaminate food crops such as cereals and nuts globally (Wild & Gong, 2010). The best-studied are aflatoxins, to which more than 5 billion people in developing countries are chronically exposed through food (Wild & Gong, 2010; Wu, Narrod, Tiongco, & Liu, 2011). Acute exposure to high levels of aflatoxin causes

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potentially fatal aflatoxicosis (Nyikal et al., 2004) and chronic exposure to naturally-occurring aflatoxins causes liver cancer (IARC, 2012). The mycotoxin fumonisin frequently co-occurs with aflatoxin in maize (Magoha et al., 2014; Mutiga, Hoffmann, Harvey, Milgroom, & Nelson, 2015; Mutiga et al., 2014; Torres et al., 2014; Wild & Gong, 2010) and chronic exposure has been associated with esophageal cancer and neural tube defects (Wild & Gong, 2010). Additionally, exposure to both mycotoxins is correlated with childhood stunting (Khlangwiset, Shephard, & Wu, 2011; Shirima et al., 2015; Wu, Groopman, & Pestka, 2014), possibly by inducing environmental enteropathy, an intestinal condition that leads to reduced absorption of nutrients (Smith, Stoltzfus, & Prendergast, 2012).

The Kenyan maize value chain, dominated by self-provisioning, purchase from open-air markets, and local milling (Hellin & Kimenju, 2009; Kang'ethe, 2011), is unable to protect consumers from foodborne exposure to mycotoxins. Aflatoxin and fumonisin are endemic in household maize supplies in Kenya (Hoffmann, Mutiga, Harvey, Nelson, & Milgroom, 2013a; Mutiga et al., 2014; Mutiga et al., 2015). Maize brought by Kenyans for local milling showed contamination above Kenyan regulatory limits of 10 ng g^{-1} aflatoxin and 1000 ng g^{-1} fumonisin in 39% and 37% of samples, respectively (Mutiga et al., 2014). Further, Eastern Kenya region has repeatedly been host to acute aflatoxicosis outbreaks shortly after the major maize harvest, including a severe outbreak in 2004 in which 125 Kenyans died (Daniel et al., 2011; Nyikal et al., 2004).

The focus of this study was to adapt a relatively-simple, multi-spectral sorter to reduce aflatoxin and fumonisin contamination in Kenyan maize. Such a device could be part of an integrated approach to mycotoxin management that empowers consumers to personally ensure food safety. Sorting exploits the fact that mycotoxin distribution is generally highly skewed: a relatively small proportion of kernels contain the majority of the toxin (Kabak, Dobson, & Var, 2006). For food-insecure populations, sorting could directly improve food security by removing the few highly-contaminated kernels in a grain lot, while retaining the majority of the healthy grain for consumption. Sorting at the individual consumer level could also help overcome the problem of misaligned incentives for mycotoxin control between producers, who often bear the costs but not the benefits of pre- and post-harvest interventions, and consumers, who are less able to demand control since the toxins are generally undetectable by human consumers (Hoffmann, Mutiga, Harvey, Nelson, & Milgroom, 2013b). This approach would represent an improvement over ineffective test-and-reject strategies that reduce an already marginal food supply, such as when 2.3 million bags of maize were condemned by the Kenyan government in 2010 due to aflatoxin contamination, and much of the contaminated maize may have been illicitly returned to the market (Ng'erich & Gathura, 2010).

Existing sorting methods to remove aflatoxins and fumonisins from maize have been summarized in larger reviews focusing on mycotoxin reduction in grains (Grenier, Loureiro-Bracarense, Leslie, & Oswald, 2013), aflatoxin detection and quantification (Yao, Hruska, & Di Mavungu, 2015), and non-biological aflatoxin remediation (Womack, Brown, & Sparks, 2014). The last review includes a table of existing applications of hand-sorting, infrared spectrometry, and ultraviolet fluorescence to the reduction of aflatoxin in tree nuts, peanuts, and maize. Low-cost spectral-sorting, such as developed in this study, was not represented. Two general approaches to sorting for mycotoxin reduction exist: sorting to remove low-quality kernels in general or sorting by algorithms calibrated to remove mycotoxin contaminated kernels specifically.

Sorting to remove low-quality, possibly fungal-infected, grains in general, which can be achieved through sieving, density separation, and removal of discolored kernels (Grenier et al., 2013). To improve maize quality, Kenyan consumers often manually sort maize using large sieve tables prior to local milling, which can be effective at reducing levels of fumonisin but may have little effect on aflatoxin levels (Mutiga et al., 2014). Alternatively traditional processing though sorting, winnowing, and washing has been shown to reduce aflatoxin and fumonisins in traditional food products in Benin (Fandohan et al., 2005, 2006). We would put into this category the 'black light' or Bright Greenish Yellow Fluorescence (BGYF) test (Grenier et al., 2013), where kernels are viewed under 365 nm ultraviolet light for fluorescence characteristic of *A. flavus* infection, specifically fluorescence of peroxidase transformed kojic acid.

Recently developed approaches use some combination of infrared, visible, and ultraviolet light imaging calibrated to detect maize kernels known to be contaminated with aflatoxin or fumonisin. Hyperspectral imaging of ultraviolet light fluorescence can classify kernels as having undetectable, low, medium, or high aflatoxin contamination (bins of <1, 1–20, 20–100, or > 100 ng g^{-1} aflatoxin, (Yao et al., 2010). Combining visible and near-infrared transmittance or reflectance spectra can classify maize by aflatoxin level (Pearson, Wicklow, Maghirang, Xie, & Dowell, 2001). Implementing this approach in high-speed sorting has been shown to reduce both aflatoxin and fumonisin contamination in maize from Texas, USA by over 80% (Pearson, Wicklow, & Pasikatan, 2004). While modern imaging approaches are effective, there is a need for improved sorting technology designed for lower-resource markets in which small samples are processed.

In this study, we calibrated a laboratory-scale, multi-spectral sorter (Haff, Pearson, & Maghirang, 2013) to remove aflatoxin- and fumonisin-contaminated kernels from diverse maize samples. Samples included maize purchased from open-air markets in Eastern Kenya and kernels from a field trial of *Aspergillus flavus*-inoculated maize. We chose to evaluate this specific sorting technology because the basic circuitry is relatively inexpensive (<US$100 in components), and throughput is modest (20 kernels/s, theoretically around 25 kg/h), providing an opportunity to adapt the design for application in small-scale milling in developing countries such as Kenya.

We tested the major hypothesis that mycotoxin levels in market maize can be significantly reduced by removing the kernels contaminated at the highest levels using a relatively simple optical sorting technology. In the process of testing this hypothesis, we also generated data on the skewed distribution of and risk factors for aflatoxin or fumonisin contamination at the single-kernel level.

2. Materials and methods

This study focused on calibrating an existing single-kernel optical sorter for the purpose of removing aflatoxin and fumonisn contaminated kernels from bulk samples of Kenyan market maize. To develop the calibration algorithms, we sourced single kernels from two concurrent mycotoxin-related studies in Kenya. Given prior knowledge that aflatoxin (Lee, Lillehoj, & Kwolek, 1980; Turner et al., 2013) and fumonisin (Mogensen et al., 2011) contamination in single-kernels is skewed, we expected aflatoxin and fumonisn contamination in our samples to also be skewed towards few individual kernels being contaminated. If we analyzed a simple random sampling of kernels from these studies, we anticipated we would not analyze sufficient contaminated kernels to develop a statistically robust calibration. Therefore, we employed multiple stages of sample selection designed to enrich for toxin-contaminated kernels in the final data set. A summary of the kernel selection process is summarized in Table 1 along with the critical analytical methods applied to each sample subset.