Efficacy of electron beam irradiation in reduction of mycotoxin-producing fungi, aflatoxin, and fumonisin, in naturally contaminated maize slurry

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1. Introduction

Maize is the main staple food crop planted mainly by small-scale farmers who account for 75% of maize producers in Kenya (Kang’ethe, 2011). Maize is commonly consumed as ugali (stiffened porridge) made from flour. Mycotoxins, especially aflatoxins (AF) and fumonisins (FUM), are a concern in the Kenyan maize industry. Between 2019 and 2021, the Kenyan Bureau of Standards (KEBS) banned more than 17 national brands of maize flour due to non-conformity to AF levels >10 ng/g (The Standard, 2020) as well as imported Ugandan maize due to FUM (>1 μg/g) non-conformity (Daily Monitor, 2021). Maize for food and feed is often contaminated with these mycotoxins in other countries, such as Germany, Poland, Belgium, Spain and Tanzania (Leite et al., 2021).

Mycotoxins are produced as secondary metabolites during fungal growth on grains either prior to harvest or during storage (Dura, 2022). Aflatoxins are produced by toxigenic members of Aspergillus section Flavi that is a group of species characterized by green yellowish conidia and dark survival structures termed sclerotia (Varga et al., 2011). Aspergillus section Flavi is composed of both aflatoxigenic and non-aflatoxigenic
species (Ehrlich, 2014). Fumonisins are produced by toxigenic *Fusarium* species. Fumonisins are produced by toxigenic *Fusarium* members Gibberella fujikuroi complex which include *F. verticillioides, F. proliferatum, F. subglutinans,* and *F. haysnianum* which express the FUM1 gene (Bluhm et al., 2004). *Fusarium* causes ear rot in maize characterized by white to pinkish scattered tufts of mold on the ears and are accompanied by starburst patterns on the kernels (Odjo et al., 2022).

Mycotoxins harm human health. The International Agency for Research on Cancer (IARC) classify AFs as class 1 carcinogens (IARC, 2002). Aflatoxins have been associated with liver cancer (Kimanya et al., 2021), retarded fetal growth in utero and infants (Alvito & Pereira-da-Silva, 2022), as well as immunosuppression (El minlawy et al., 2014). Aflatoxins in animals have been linked to reduced productivity, increased incidence of diseases, damage to vital organs, and death (Robens and Richard, 1992). Furthermore, AFs cause $750 million loss annually in Africa (Gbashi et al., 2018). Fumonisins are associated with esophageal (Khan et al., 2019), neural tube defects (Gelineau-van Waes et al., 2009), and liver cancer in humans (Al-Khalidi and Jabbar, 2019). They are class 2 carcinogens (IARC, 2002).

Reducing mycotoxins in food value chains calls for an integrated approach, involving pre-harvest and post-harvest interventions (Lin et al., 2022). Ionizing irradiation using gamma (Zhang et al., 2018) or electron beam (eBeam) (Woldemariam et al., 2021) is one potential intervention for reducing mycotoxins. To the best of our knowledge, Woldemariam et al. (2021) is the first report of work evaluating eBeam for an application in Sub-Saharan Africa – there to reduce aflatoxin (unsuccessfully) and ochratoxin A (successfully) in red pepper from Ethiopia, with treatment done in Germany. Ionization treatment using gamma radiation and eBeam reduces both *Asterigillus* and aflatoxin B1 in Brazil nuts (Assuncão et al., 2015). Gamma irradiation eliminates fungi in soybean (Zhang et al., 2018).

These potential effects of eBeam on mycotoxins and fungi are a specific case of the general potential for ionizing technologies to have significant value for enhancing food safety, enhancing shelf life, and preventing post-harvest spoilage of agricultural products (Lung et al., 2015; Pillai & Shayanfar, 2017, 2018; Woldemariam et al., 2021). The mechanism of action of ionizing technologies is either through direct action, where energetic photons or electrons directly cause covalent and hydrogen bond breaks, or indirectly, where the products from the radiolytic splitting of water molecules indirectly cause extensive covalent and hydrogen bond breaks (Khaneghah et al., 2020). The US FDA currently approves doses up to 30 kGy for spices and food ingredients (Komolprasert et al., 2022).

This study investigated the efficacy of eBeam in degradation of naturally occurring aflatoxins and fumonisins, as well as inactivating the fungi, *Asterigillus* and *Fusarium,* that produce them. This was to explore how eBeam irradiation may help co-manage multiple mycotoxins in a naturally contaminated staple food.

2. Materials and methods

2.1. Sample collection and processing

As part of on-going work at the International Institute of Tropical Agriculture, 1 kg shelled maize kernel samples were collected from farmers’ stores in Upper Eastern Kenya counties: Embu, Meru and Tharaka Nithi Counties. 885 samples were collected and shipped in cooler boxes to the Regional Mycotoxin lab in Machakos County, Kenya. The samples were ground in a lab mill grinder and analyzed for AFs using a commercial lateral flow assay (Accuxcan Reveal Q – Pro-reader, Neogen, USA). From these 885 samples, 100 g of the 97 samples which contained AFs greater than 10 ng/g were shipped to the University of Illinois for irradiation studies (under USDA APHIS import permit to move live plant pests, noxious weeds, and soil, PS26P-21-00992_20210303). While samples were already sun dried by the farmers before storage, those that appeared wet were further oven dried at 45 °C for 48 h to stabilize them before shipping to Illinois in flour form. The focus of this specific study was not a survey of mycotoxin in farmer’s stores, and so the data for the full 885 samples is not relevant here. Instead, our study focused on the effect of irradiation. We selected highly contaminated samples to ensure there would be sufficient mycotoxin present to show an effect of irradiation if one occurred.

2.2. Bulk aflatoxin and fumonisin analysis in maize flour

The 97 samples were manually shaken to mix and analyzed for AF and FUM using commercially available, manufacturer-validated, ELISA methods (Total AF ELISA Quantitative and FUM ELISA Quantitative, Helica Biosystems Inc., Santa Ana, CA). Five grams of each sample were mixed with 25 ml of 80% methanol and shaken for 3 min in an orbital shaker (Thermo Scientific MaxQ 4450 Orbital Shaker Incubator, Florida, USA). The kit manufacturer’s protocol was followed for testing. Samples that tested higher than the detection limit were further diluted in 80% methanol and reanalyzed. For FUM, the samples were further diluted in distilled water to attain a 1:40 dilution sample to methanol ratio. Both AF and FUM levels were calculated by comparison to standard curves generated for each individual ELISA plate. Individual plates standard curves showed R² values of 0.99 for standards ranging from 0.0 to 4.0 ng/g AF and for standards ranging from 0.0 to 6.0 pg/g FUM, validating the kits were used correctly. The 24 highly contaminated maize samples that had both >100 ng/g of AF and >1000 ng/g of FUM were selected for the eBeam dosing studies.

2.3. Preparation of maize slurry and subsampling

Ten grams of the 24 selected highly contaminated samples were each mixed with 50 ml of sterile de-ionized water and shaken for 3 min. We used a maize slurry matrix to simulate a common maize food matrix that is consumed in Kenya and because efficacy of eBeam in degradation of mycotoxin is likely higher in aqueous medium. Each sample was sub sampled into four units for treatment with one of the four irradiation doses. Approximately 7 ml of slurry was pipetted into sterile, plastic sample bags (small Whirl Pak, VWR, USA), double bagged, and heat-sealed in 95 kPa Transport Bags (Therapak, VWR, USA) for eBeam dosing treatment at Texas A&M University. The samples were stored in a cold room at 8 °C and shipped overnight with frozen ice packs.

2.4. eBeam dosing

The eBeam dosing was performed using a 10 MeV, 15 kW S-band linear accelerator at Texas A&M University’s National Center for Electron Beam Research. The samples, still in the flat, heat-sealed bags from shipping, were placed on an automated conveyor system and exposed to the high energy electrons. Samples were treated at target doses of 5, 10, and 20 kGy as shown in Table 1. Doses were measured by using alanine dosimeters. The alanine dosimetry used in this study was traceable to international standards. The alanine dosimeters were read using a

| Table 1 |
|---|
| Dose uniformity during slurry treatment in sterile plastic bags. |
| Intended dose (kGy) | Measured dose (kGy) | Dose location | Dose Uniformity ratio (Max/Min Dose) |
|---|---|---|---|
| 5 | 4.769 | Top | 1.02 |
| 10 | 9.496 | Top | 1.02 |
| 20 | 20.407 | Top | 1.00 |

*Measured by alanine dosimetry.*
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Buker e-Scan EPR spectrometer. The doses were calibrated using the product conveyor speeds. The dose rate of the 10 MeV, s-band 15 kW linac was approximately 3 kGy/s.

Preliminary experiments were performed to ensure that the packaged samples would receive uniform doses. Ensuring dose uniformity is critical when performing such ionizing technology dosing treatments (Espinosa et al., 2011; Praveen et al., 2013). Critically, the dose uniformity ratios of 1.00–1.02 between the top and bottom of the bags demonstrates that eBeam penetration depth was not a source of error in these experiments – full doses were delivered to the samples. A control, non-irradiated, sample (0 kGy) was also included to measure effects of shipping samples between Illinois and Texas.

2.5. Microbial analysis

2.5.1. Microbial isolation

After eBeam dosing samples were shipped overnight University of Illinois for microbial isolation. A 1 ml aliquot of each control and irradiated maize slurry was serially diluted in phosphate-buffered saline. Then 50 μl aliquots were plated on Potato Dextrose Agar (PDA) complemented with 2.5 ml/L streptomycin and chloramphenicol and incubated at 31 °C for 5 days and viable fungi counts were enumerated (Garber et al., 2012).

2.5.2. Molecular identification

All control slurry sub-samples were analyzed for presence of Aspergillus and Fusarium-specific DNA signatures. DNA extraction was done from four sources: (i) pure cultures of Aspergillus flavus S-strain, (ii) Fusarium proliferatum isolated from AF and FUM maize samples using PDA, (iii) pure uncontaminated maize, and (iv) contaminated maize slurry samples which were not irradiated.

Aspergillus and Fusarium DNA was extracted from pure cultures to create standard curves for qPCR using the method of Mideros et al. (2009). Pure cultures of Aspergillus flavus S-strain and Fusarium proliferatum were grown on PDA for 5 days and the plates were washed with 5 ml of Glucose Yeast Extract Peptone (GYEP) broth and transferred into sterile plates 8 ml of GYEP broth. The GYEP broth plates were incubated at room temperature for 48 h. After incubation the broth was centrifuged, the pellet rinsed with sterile deionized (DI) water, and the pellet resuspended in 1 ml of DI water for DNA extraction using IBI kit protocol. Extracted DNA was quantified in ng/μl using a Nano drop (2000C, Thermo Fisher Scientific, USA). The purity of the DNA was also determined by 260/280 ratio greater than 2.0. These high-purity Aspergillus and Fusarium DNA were later diluted and used for qPCR standard curves.

DNA was also extracted from uncontaminated maize flour and contaminated maize slurry samples, using an IBI extraction kit protocol (IBI Scientific, Iowa, USA). The uncontaminated maize sample control had pre-tested negative for Aspergillus and Fusarium infection by qPCR (methods described below) and negative for aflatoxin and fumonisin by ELISA. Serial dilutions were done for the pure cultures of pathogen DNA where 10, 1, 0.1, 0.01, and 0.001 ng/μl was mixed with 1 ng/μl of pure maize DNA to make a mixed standard curve to represent the matrix of the test sample (fungal DNA in maize background). For maize test samples, 100 mg of flour slurry sample was used for DNA extraction.

Quantitative PCR was performed in a Q-machine (model 95,900-4C, Quantabio, MA, USA) according to Mideros et al. (2009) for detection of Aspergillus species. The internal transcribed spacer 1 (ITS1) primers and Taqman probes were obtained from Life Technologies Corporation, USA: Forward primer 5′-ATCCATTACCGAGTGTAGGGTTCCT-3′; reverse primer: 5′-GCCGAAGAACAGGAGTGTTAGGCTTCT-3′; and TaqMan probe was 5′-FAM-CGAGCCAACCACCCAGC-3′ TAMA, which resulted in 73 bp amplicon. The 25 μl reaction mix (1X) was prepared by using 12.5 μl of TaqMan Fast Advanced Master Mix (Life Technology Corporation, USA), 1.9 μl of 10 μM of each forward and reverse primer, 0.2 μl of 25 μM probe and 5.5 μl Nuclease free PCR water and 3 μl of template DNA. The reaction was conducted with a two-profile pre-cycle of 50 °C for 2 min; 95 °C for 10 min followed by 40 cycles of 95, 59, and 72 °C for 30 s each.

For Fusarium the qPCR was done according to Bluhm et al. (2004). ITS primers and probes were obtained from Life Technologies Corporation, USA: ITS forward- 5′-AATCTCCAAACCCCTGTGAACATA-3′; ITS reverse- 5′-TTTACCGGCTGCGCCGC-3′ and ITS Taqman probe 5′-CGTCGAACAGGAGTCGGCCGAATAC-3′TAMRA which resulted in a 431 bp amplicon product. The 20 μl reaction mix (1X) was prepared by using 10 μl of TaqMan Fast Advanced Master Mix (Life Technology Corporation, USA), 1 μl of 10 μM of each forward and reverse primer, 0.1 μl of 20 μM probe and 2.9 μl Nuclease free PCR water and 5 μl of template DNA. The reaction was conducted with a two-profile pre-cycle of 50 °C for 2 min; 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 2 min each.

The amount of pathogen DNA in the sample was estimated by using the mixed standard curves equations. For Aspergillus flavus DNA in maize DNA the equation was $y = -3.50x + 20.1$ and efficiency of the reaction was 93%, R = 0.99. For Fusarium proliferatum DNA the equation was $y = -3.38x + 21.1$ with an efficiency of 98%, R = 0.99. The MIQE checklist was used to include the essential information (Bustin et al., 2009). The infection coefficient (IC) was calculated as the proportion of fungal pathogen DNA over the total DNA in the sample and was used to estimate the pathogen proportion in the sample.

2.6. Aflatoxin and fumonisin analysis from treated slurry

A 1 ml aliquot of the each of the 24 maize slurry samples that were eBeam-treated, and controls, were mixed with 5 ml of 80% methanol. These extracts were homogenized by vortexing and analyzed for FUM and AF using procedures described earlier for ELISA analysis.

2.7. Data analysis

R software version 4.1.1 was used for data analysis. AF, FUM, and microbial counts data were log 10 transformed. This is typical for mycotoxin data which typically show significant right tail skewness (Stasiewicz et al., 2017) and standard practice for microbiological counts data where fungi replicate and die off exponentially. Pearson correlation coefficient was used to determine the relationship between the fungal populations and the mycotoxins present in the samples. Mycotoxin levels in each sample was estimated using the standard curve developed in each plate using ELISA standards. This was determined using quantitative PCR analyses. The IC values were also log transformed. Student t-test with False Discovery Rate correction was used to determine whether the log mean difference of the control and eBeam treated samples’ toxin levels were greater than zero – individual tests were performed for each of 6 conditions (AF or FUM, 3 doses each) and p-values adjusted using the p. adjust function in R. All raw data and analytical code in R were posted to GitHub (https://github.com/ioo-dasfetlab/Mohamed-2022-Ebeam).

3. Results

3.1. Aflatoxin and fumonisin were not significantly correlated in samples selected for high aflatoxin level for eBeam processing

Eighty-two samples out of 97 had AF level higher than the KEBs threshold (>10 ng/g) (Table 2 and Supplementary Fig. S1). The AF levels ranged between 1.3 and 1290 ng/g. The average AF level was 1.64 log (ng/g) with a standard deviation of 0.63. For FUM, 57 out of the 97 samples had levels greater than the KEBs threshold (>1000 ng/g). The FUM levels in the samples ranged between below detectable levels to 11,600 ng/g. The average fumonisin level in the 97 samples was 3.4 log (ng/g) and high FUM (>10 ng/g) and high FUM (>1000 ng/g). Twenty-
Table 2
Mycotoxins level in highly contaminated flour samples (n = 97).

| Mycotoxin | Threshold (ng/g) | No. of samples | Levels of mycotoxin (ng/g) | Average | Median | Ranges |
|-----------|------------------|----------------|---------------------------|---------|--------|--------|
| Aflatoxin | below KEBS (<10) | 15             | 5.7                       | 5.8     | 1.3-9.7|
|           | above KEBS (>10) | 82             | 130                       | 66      | 10.2-1290|
| Fumonisin | below KEBS (<1000) | 40            | 254                       | 153     | 0.936  |
|           | above KEBS (>1000) | 57             | 3580                      | 2940    | 1000-11600|

* As per method section 2.1, these 97 samples were selected out of a pool of 885 samples based on an AF measurement >10 ng/g by the field team in Kenya that collected the samples. Some of these samples tested AF < 10 ng/g (but with detectable aflatoxin) upon receipt at Illinois, which is not unreasonable given the known skewness of aflatoxin contamination.

Four samples that had AF level (>100 ng/g) and FUM level (>1000 ng/g) were chosen for eBeam dosing. AF and FUM levels did not show significant correlation (r = 0.097, p = 0.34) (Supplementary Fig. S1).

3.2. Populations of Fusarium spp. were higher than Aspergillus in samples

The viable fungal populations in the control sample based on PDA counts ranged between 2.6 and 4.6 log (CFU/g) with an average of 3.9 log (CFU/g) and standard deviation of 0.53 log (CFU/g). Quantitative PCR assay showed the reaction efficiency of 93 and 98% for Aspergillus and Fusarium respectively (Supplementary Fig. S2). The average infection coefficient (IC) of maize with Fusarium (−0.3 log) was higher than infection with Aspergillus (−2.8 log). The IC with Fusarium ranged between −1.89 and 0.75 log (CFU/g) while for Aspergillus it ranged between −4.08 and −2.09 log (CFU/g) (Fig. 1). There was a significant difference between the IC of Fusarium and Aspergillus (t = 19.4, p < 0.001). There was a significant positive correlation between the IC of Fusarium and Aspergillus (r = 0.5, p = 0.03).

Fig. 1. Infection coefficients in log scale (pathogen/Total DNA ratio) of total Aspergillus (n = 24) and Fusarium (n = 24) DNA in maize DNA. Box plot represents the ratio (IC) of the fungi estimated by TaqMan quantitative real-time polymerase chain reaction (qPCR) and total DNA estimated by NanoDrop in ng/µl. Means of Aspergillus and Fusarium IC were significantly different (t = 19.37, p < 0.001).

3.3. Total microbial load positively correlated with mycotoxins levels in slurry

There was a significant positive correlation (p = 0.03, r = 0.54) between the IC with Aspergillus and the AF level. A similar trend was seen with IC of Fusarium and FUM in the samples (p < 0.001, r = 0.68) as shown in Fig. 2. The AF levels in the slurry sample ranged between −0.6 and 2.0 log (ng/g) while the Aspergillus IC ranged between −4.1 and −2.1 log. Fumonisin levels ranged from 1.0 to 3.9 log (ng/g) while the Fusarium IC ranged between −1.9 and 0.75 log. As will be discussed in section 4.1, these data correlating fungal biomass and mycotoxin levels were a relatively unique contribution of this study, even though these data are secondary to the study’s main focus on effects of ebeam irradiation.

3.4. eBeam was effective in reducing fungal load in the slurry

Exposure to even 5 kGy eBeam dose reduced all fungi to below detectable limits (LOD of 1.9 log (CFU/g)). Results were similar for higher doses of 10 and 20 kGy (Supplementary Table S1). Based on measured initial populations, the efficacy of 5 kGy dose ranged between >0.7 and >2.7 log (CFU/g) reduction, with an average reduction of >1.8 log (CFU/g).

3.5. eBeam was effective in reducing aflatoxins at 20 kGy

Treatment of AF contaminated maize slurry with 20 kGy resulted in an average 0.3 log (ng/g) reduction in AF (False Discovery Rate (FDR) corrected p = 0.03) (Fig. 3). The reduction ranged from −0.7 to 1.4 log reduction. Sixty one percent of the samples showed reduction at 20 kGy. Seven out of ten samples that had >1 log (ng/g) of AF were reduced after 20 kGy treatment. There was no significant change (0.03 and −0.1 log reduction) in AF levels at 10 and 5 kGy dose treatment respectively as shown in Supplementary Figs. S3 and S4.

Here authors note that some treated samples measured greater toxin levels than untreated samples (a negative reduction, i.e., increase). Such variation in treatment effects have been reported in other remediation
studies (Stasiewicz et al., 2017). This is most likely due to random chance. One consequence of working with mycotoxins, which have highly variable levels within samples, is that variation within a sample can be larger than the biological effect of a treatment. Then, in cases like these where one must test different physical sub-samples before and after treatment, it is possible the ‘after’ sample randomly had a much higher value than the ‘before’ sample, higher than the effect of eBeam treatment to reduce. For a sense of sub-sample variability, one could refer to the 5 kGy treatment (Supplemental Fig. S4) which also shows some sample, with ‘after’ levels higher than ‘before’ even though there is no effect of eBeam. There is no scientific evidence, nor plausible biological mechanism, that eBeam treatment itself would increase mycotoxin level.

3.6. Fumonisin was resistant to eBeam degradation even at 20 kGy

After treatment with 5, 10, and 20 kGy doses (Fig. 4, Supplementary, Figs. S5 and S6 respectively), there was no significant reduction (FDR corrected p > 0.05) in FUM. Average reductions of 0.005, 0.01, 0.2 log (ng/g) were seen at 5 kGy, 10 kGy and 20 kGy respectively.

4. Discussion

4.1. Pre-formed mycotoxins are positively associated with fungal biomass in maize slurry

Our results indicate positive correlation between the infection coefficient of the fungi and the mycotoxin levels in the samples i.e., Aspergillus spp. and measured AFs and Fusarium spp. and measured FUMs in the slurry samples. Mideros et al., 2009 found a similar significant correlation (r = 0.85) between Aspergillus biomass and AFs levels in field inoculated maize. A positive correlation (r = 0.85) has also been shown between FUMs and Fusarium inoculated wheat (Siou et al., 2014). To the best of our knowledge, this study is novel in reporting correlation...
between fungal biomass by qPCR and measured toxin levels in naturally contaminated maize samples.

Other studies that involve naturally contaminated samples use traditional culture-based plating methods. They recovered a high proportion (97.9%) of fungal biomass by qPCR and measured toxin levels in naturally contaminated maize samples. This further mycotoxin production is not a trait expressed by all fungi. Aflatoxin production has been associated with expression of Nor-1 gene (Iheanacho et al., 2014) in toxigenic Aspergillus. The inability of atoxigenic members of Aspergillus section Flavi to produce AF is due to deletion in genes involved in AF biosynthesis (Probst et al., 2014). Aspergillus flavus and A. parasiticus are the most implicated members of Aspergillus section Flavi that are involved in AF production (Probst et al., 2007). Fusonins similarly are produced by toxigenic members of Fusarium that express the FUM gene (Bluhm et al., 2004).

For food security, if the samples are improperly stored, typically too wet, warm and exposed to external environment, the presence of the toxigenic fungi in the maize poses a threat of further toxin production and accumulation (Darwish et al., 2014). This will result in greater economic burden for the farmers and millers. The product with the toxigenic fungi may provide a source of inoculum for infecting other products if stored and processed in the same lots, which may reduce marketable volumes. Furthermore, mycotoxins affect trade in local food processing industries that acquire their raw materials from small holder farmers whose product may contain high levels of toxins resulting into further mycotoxin production which may increase shelf life by preventing further fungal proliferation (which could decrease spoilage) and prevent toxin production and accumulation (which would ensure safety). Additionally, eBeam has a potential in reduction of microbes along other food value chains which include vegetables, fruits, meat products (Khaneghah et al., 2020) and spices (Gryczka et al., 2020). Globally, more than 60 countries have approved use of irradiation in food. Different countries have different regulations on the maximum irradiation dose applied on food (Freitas-Silva et al., 2014). 10 kGy is the recommended dose that cause no physical chemical alteration to the food matrix. However, Irradiation doses as low as 1 kGy are being used in pepper, persimmon, tomatoes and tropical fruits and these doses as high as 30 kGy have been authorized for herbs and spices (Freitas-Silva et al., 2014).

4.2. eBeam eliminated viable fungal populations which may reduce further mycotoxin production

In the current study, eBeam doses as low as 5 kGy reduced fungi below detection limits in naturally contaminated maize slurry. This finding concurs with earlier studies that illustrated 5 kGy eBeam dose was effective in causing a 3 log reduction of coliphage, total coliforms and total flora in waste water (Farooq et al., 1993); total microbial load in turmeric powder (Esmaili et al., 2018) and Aspergillus at 4.8 kGy in maize (Nemt ¸ anu et al., 2014). A slightly higher dose of 6 kGy of eBeam has been shown to be effective in inactivating fungi spores (Etter et al., 2018). Other studies which evaluated a higher dose of 10 kGy also found the technology to be efficacious in soybean (Zhang et al., 2018). A recent study indicated that even a slightly lower dose (4 kGy) was effective in reducing yeasts by 2.0 logs and other fungi by 3.4 logs in red pepper (Woldemariam et al., 2021). Sensitivity of fungi to irradiation is dependent on stage of fungal infection present in the sample (hyphae vs spores); moisture content of spores/matrix; age of spores; pretreatment of the sample i.e., freezing or heating (Calado et al., 2014). Radiosensitivity also differs from one species to another, Penicillium are more sensitive than Fusarium while Fusarium is more sensitive than Aspergillus species when exposed to eBeam irradiation (Nemt ¸ anu et al., 2014).

For food safety and security, elimination of fungi in flour samples may increase shelf life by preventing further fungal proliferation (which could decrease spoilage) and prevent toxin production and accumulation (which would ensure safety). Additionally, eBeam has a potential in reduction of microbes along other food value chains which include vegetables, fruits, meat products (Khaneghah et al., 2020) and spices (Gryczka et al., 2020). Globally, more than 60 countries have approved use of irradiation in food. Different countries have different regulations on the maximum irradiation dose applied on food (Freitas-Silva et al., 2014). 10 kGy is the recommended dose that cause no physical chemical alteration to the food matrix. However, Irradiation doses as low as 1 kGy are being used in pepper, persimmon, tomatoes and tropical fruits and these doses as high as 30 kGy have been authorized for herbs and spices (Freitas-Silva et al., 2014).

4.3. eBeam was efficacious in reducing preformed mycotoxins

eBeam at 20 kGy was found to significantly reduce total AF in the naturally contaminated maize slurry while no significant effect was seen with dose of 10 kGy and below. This may be attributed to the high dose of eBeam that had a higher chance of causing degradation of the AF in...
the water medium of the slurry. Efficacy of eBeam on mycotoxin is influenced by the moisture content of the sample, the higher the water content the higher the direct degradation effect (Liu et al., 2016).

Degradation also occurs indirectly in higher moisture concentration as the water molecule is broken down into hydroxides and hydrogen ions which increase degradation of the mycotoxin (D’Ovidio et al., 2007). Degradation products of mycotoxins after eBeam irradiation have been shown to have a reduced mutagenic and cytotoxic effect compared to the mycotoxins in original form (Liu et al., 2016). In a recent study, doses of up to 30 kGy of eBeam were evaluated and found to be ineffective in reducing AF but significantly reduced ochratoxin A by 25% in dry chili pepper (Woldemariam et al., 2021). Efficacy of other irradiation techniques, like Gamma, in degrading mycotoxins, is also affected by moisture content of the matrix. In a study by Julii et al. (2012), Gamma dose of 30 kGy applied to pepper at 18% moisture content resulted in a 55.2%, 50.6%, 39.2%, 47.7% and 42.9% for ochratoxin A, AF B1, AF B2, AF G1 and AF G2, respectively. Therefore, higher doses of up to 50–100 kGy have been suggested for complete degradation of mycotoxins (Temcharoen and Thilly, 1982). However, other studies have shown eBeam at a lower dose of 10 and 15 kGy was efficacious in reducing AF B1 in particular in Brazilian nut (Assuncao et al., 2015) and beans (Supriya et al., 2014).

5. Conclusions

EBeam irradiation treatment eliminates both Aspergillus and Fusarium in maize slurry. This suggests treatments of these products could eliminate the risk of further mycotoxin formation if they had to be held for extended periods at warm temperatures. In addition, the fact that a high dose of irradiation did reduce aflatoxin in slurry suggest a possibility to use this technology to at least partially remediate contaminated product, particularly if economic or food security pressures make it unreasonable to destroy contaminated maize. Since fumonisin was not reduced by up to 20 kGy of eBeam irradiation, this technology may not be useful to reduce pre-formed fumonisin or may require much higher doses to have a meaningful effect.

Author credit statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All raw data and analytical code in R were posted to GitHub (https://github.com/foodsafetylab/Mohamed-2022-Ebeam).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxix.2022.100141.

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