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Assessment of aflatoxin levels in whole and peeled ginger marketed in Dar es Salaam, Tanzania

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ABSTRACT

Aflatoxins are naturally occurring toxins, predominantly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins are often found in numerous agricultural and food products worldwide. The current study investigated the natural occurrence of total aflatoxins in dried split ginger purchased from different local markets in the Dar-Es-Salaam region, the eastern zone of Tanzania. Using a cross-sectional sampling design, a total of 50 ginger samples were analyzed in two preparations as a whole and peeled ginger based on Q+ Aflatoxin single-step lateral flow immuno-chromatographic assay. The results revealed that all ginger samples analyzed were positive for aflatoxins with levels ranging from 5.7–28.0 μg/kg and 3.1–21.5 μg/kg for whole and peeled ginger respectively. Forty (80%) and fifteen (30%) of the samples for whole and peeled ginger respectively were above the Tanzanian legally permissible limit of 10 μg/kg. Interestingly, aflatoxins level of contamination in whole ginger were significantly higher than in peeled ginger ($p<0.0001$). This is the first report on the natural occurrence of aflatoxins in whole and peeled ginger samples from Tanzania. The findings of this study indicated that ginger peels are relatively prone to aflatoxin contamination.

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1. Introduction

Agricultural products including spices are usually prone to contamination at different stages of harvesting, handling, storage, and distribution by some species of fungi that are responsible for spoilage and production of aflatoxins. Therefore, improper storage, drought, and high humidity enhance the growth of aflatoxigenic molds in herbs and spices (1,2).

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Ginger is known to possess medicinal properties, as well as having antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects against a few gram-positive and gram-negative bacteria (3). Based on these health benefits, ginger has a very long history of use as a traditional and alternative medicine.

Aflatoxins are toxic molecules that are produced predominantly by fungi *Aspergillus flavus* and *Aspergillus parasiticus*. A substantial percentage of

agricultural products including ginger worldwide are contaminated by aflatoxins of which significant ones namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (4).

Reduction of mycotoxin contamination can be achieved by several processes including cleaning, milling, brewing, fermentation, baking, nixtamalization, and hulling of grains. In particular, the removal of outer layers of grains and some spices, known as de-hulling techniques, has been shown to minimize mycotoxin contamination (5,6). Furthermore, dry milling was found to be an effective way of reducing aflatoxin levels in contaminated grain. The uppermost levels of aflatoxin B1 after the process of milling were obtained in the hull fractions of corn. Similar studies in rice and wheat also showed that the bran had a higher concentration of aflatoxin (7). On the other hand, the restriction of fungal colonization and mycotoxin accumulation to shell layers of the kernel were the prerequisites for the success of de-hulling in toxin content reduction (8). For example, a de-hulling process in maize was found to remove up to 93% of aflatoxins (9). Similar to other regions of the world, ginger is increasingly popular in the Tanzania market as one of the important spices. Studies were mainly limited to knowledge, awareness, and practices on aflatoxin contamination of staple foods such as maize, peanuts, and animal feeds (10-14). Few studies in Tanzania reported detection of total aflatoxin contamination in whole spices (15). Therefore, in this study, we aimed to analyze total aflatoxin contamination in entire and peeled gingers. The primary hypothesis was that ginger peels were likely to be more contaminated with aflatoxin-producing fungi than peeled ginger. As expected, the degree of fungal colonization into different fractions of grains or tuber crops is reflected in the redistribution of aflatoxins. Thus, high aflatoxin concentrations are anticipated on the surface tissues of grains and tuber crops.

2. Materials and Methods

2.1. Study area and sample collection

The study was conducted in the Dar-Es-Salaam region, eastern zone of Tanzania with temperatures ranging between 23–31°C in summer and 21–28°C in winter. The average total annual rainfall is approximately 734 mm, with most of it falling during March–May. Using the cross-sectional sampling method, 50 samples of locally cultivated ginger (Zingiber officinale) were randomly collected from different local markets in Dar-Es-Salaam.

2.2. Sample preparations and analysis

All samples were analyzed in two preparations, the whole ginger and peeled ginger using Q+ Aflatoxin (Neogen Corporation, Michigan, USA) as described by Lee and others (16). Briefly, a representative sample was ground and weighed out of 10 g. 50 ml of 65% ethanol was added to a sample and shaken vigorously for 3 min. The mixture was allowed to settle and filtered and 3 ml was collected as a filtrate. Then, 100 µl of the sample extract was added into a red dilution cup and mixed thoroughly 5 times. Thereafter, 500 µl of sample diluent was added into the red dilution cup and 100 µl of the diluted sample extract was transferred into another sample cup. A new Reveal Q+ Aflatoxin test strip was placed into a sample cup and a timer was set for 6 min. Finally, a test strip was removed and placed into the Reveal AccuScan Gold reader (Neogen Corporation, Michigan, USA). The results were read in

10–20 s, showing aflatoxin levels in ppb. The lower limit of detection (LOD) of the assay was 2.2 ppb, with a quantitative range of 2.2 to 29.0 ppb. The minimum and maximum contamination levels were 3.1 and 28.0 ppb, respectively. Results over 100 ppb were diluted and re-run. All ginger samples that were collected and processed were analyzed in duplicate.

2.3. Statistical analyses

Data were tested for normality by using the Ryan-Joiner (similar to the Shapiro-Wilk) test. The findings were given as mean levels using standard deviations. To evaluate the significant difference among the aflatoxins in whole and peeled ginger samples, paired T–test ($\alpha = 0.05$) was used. All statistical analyses were conducted by using Minitab Statistical Software version 21.0 for Windows (Minitab Inc., Shanghai, China).

3. Results

3.1. Total aflatoxins quantification using Immuno-chromatographic assay

In this study, total aflatoxins were screened in ginger samples using the immuno-chromatographic assay, with the results being summarized in Table 1, which presents the occurrence of total aflatoxins. This indicates that all the screened whole and peeled ginger sample types contain aflatoxins in the range between 5.7–28.0 ppb and 3.1–21.5 ppb respectively. The mean values were 16.42 ppb and 9.24 ppb respectively for whole and peeled ginger.

3.2. Variation of aflatoxins concentration between whole and peeled ginger

A significant difference in Aflatoxins levels between the two sample types was found; whereby Aflatoxins levels in whole ginger were significantly higher than in peeled ginger ($p<0.0001$). Likewise, Aflatoxins contamination levels were relatively higher in all whole ginger samples as compared to peeled ginger (Figure. 1).

Table 1. Detection of total aflatoxins by Immuno-chromatographic assay

Sample type	% of Contamination	Range (ppb)	Mean ± SD (ppb)
Whole ginger	100	5.7–28.0	16.42 (± 5.95)
Peeled ginger	100	3.1–21.5	9.24 (± 4.26)

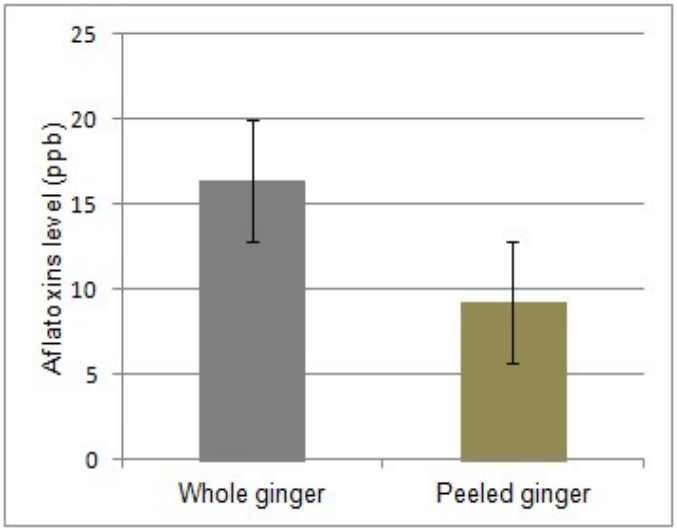

Figure 1. Occurrence of total Aflatoxins (ppb) in whole ginger and peeled ginger

4. Discussion

To the best of our knowledge, this is the first study to investigate aflatoxin contamination in whole and peeled ginger in Tanzania. All ginger samples collected from local markets in Dar-Es-Salaam were contaminated with aflatoxins. We found that 80% and

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30% of the samples for the whole and peeled ginger respectively were contaminated with aflatoxins which exceeded the Tanzanian legally permissible limit for food stuffs. The occurrence of aflatoxins in ginger has been previously reported. In agreement with our findings, another study conducted in Dar-Es-Salaam, Tanzania which surveyed cinnamon, ginger, cloves, and cardamom found that the total aflatoxin contamination ranged from 0.55–9.66 µg/kg in seventeen (56.7%) ginger samples (15). A study in Nigeria showed that sixty-six ginger samples (55%) were contaminated by aflatoxins at levels up to 9.52 µg/kg (17). Similarly, in South Africa, a comparative study of aflatoxin contamination of winter and summer ginger found that all samples were contaminated at a rate higher than those recommended by the European Union (EU) (3). On the contrary, even though in India it was reported that all forty (100%) ginger samples analyzed were contaminated with aflatoxin they were below the limit level of 10 µg/kg (18).

Furthermore, we have noted a significant difference in the levels of aflatoxins between the two sample types, whereby aflatoxin levels in whole gingers were higher than in peeled gingers. This indicated that the highest levels of aflatoxin contamination were found in ginger peels, (7,19) reported significant amount of aflatoxins were found in hull and bran fractions of maize and rice respectively that were processed by dry milling. They further highlighted that dry milling is effective in fractionating the aflatoxins found in contaminated grains, and this method can be a cost-effective way of minimizing aflatoxin levels in the primary products, such as flour. Similarly, de-hulling of maize and barley was found to remove over 80% of the aflatoxins and Deoxynivalenol levels (20,21). A similar finding was obtained in another study investigating total aflatoxin and fumonisin levels in marketed maize and maize products in Tanzania. The total aflatoxin and fumonisin levels were significantly higher in maize bran fractions as compared to maize milled and flour fractions (22). Apart from de-hulling and dry milling, other several physical methods can be employed to reduce aflatoxin contamination in cereals and spices. Sorting and washing are the commonest methods to remove broken and damaged kernels, which usually contain the majority of the aflatoxins contamination (5). Another study reported that using a combination of cleaning, polishing, and removal of the bran and offal fractions, they observed an overall reduction of about 75% of Ochratoxin A in white bread (23). Our results suggested that the physical removal of ginger peels may significantly reduce the natural aflatoxin contamination. While it has been established that physical methods of food processing and preservation are effective in reducing the growth of molds and aflatoxin production, the use of antagonistic yeasts or probiotic yeasts also showed great promise in preventing aflatoxin production (24,25). A previous study demonstrated that the use of antagonistic yeast such as *Kluyveromyces lactis* in food preservation significantly inhibits mycelia growth of *A. parasiticus* and the production of several types of aflatoxin (26). Apart from physical methods of food processing and the use of antagonistic yeasts in reducing aflatoxin contamination, the heating process have been shown to effectively minimize aflatoxin levels in food and feed products. This is based on the fact that the levels of aflatoxins in foods have been shown to correlate with water activity and temperature (27). A study found that moisture content was minimized by over 91% in all

food samples that were heated with a concomitant reduction in aflatoxin B_1 concentration to the levels permissible to international limits (28).

Although ginger is a common spice used globally, it is recognized as a significant carrier of aflatoxins mainly because it is a root plant. Substandard preparations after the harvest and along the retail chain, mostly in developing countries, may pose the risk of contamination. Food processing including physical preparations such as cleaning, peeling, and scouring are known to reduce mycotoxin exposure and contamination.

5. Conclusion

This study investigated the natural occurrence of aflatoxins in whole and peeled gingers sourced from local markets in the Dar-Es-Salaam region of Tanzania. We found all the gingers collected and analyzed were contaminated with aflatoxins, 80% of whole ginger and 30% of peeled ginger respectively exceeded the Tanzanian legally permissive limit of 10 mg/kg, underlining the potential health effects on humans in the study area. To minimize the health risks of aflatoxin exposure and contamination, we recommend physically removing ginger peels as an initial stage of food processing before human consumption.

Conflicts of Interest

The authors declare no conflicts of interest in this manuscript.

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