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## Research Note

# Study of an Educational Hand Sorting Intervention for Reducing Aflatoxin B<sub>1</sub> in Groundnuts in Rural Gambia

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## ABSTRACT

Aflatoxin, a human liver carcinogen, frequently contaminates groundnuts, maize, rice, and other grains, especially in Africa. The aim of this study was to evaluate the effectiveness of an educational intervention that involved training rural Gambian women on how to identify and remove moldy groundnuts to reduce aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) contamination. In total, 25 women, recruited from the West Kiang region of The Gambia, were trained on how to recognize and remove moldy groundnuts. Market-purchased groundnuts were hand sorted by the women. Groundnuts were sampled at baseline ( $n = 5$ ), after hand sorting (“clean,”  $n = 25$  and “moldy,”  $n = 25$ ), and after roasting ( $n = 5$ ). All samples were analyzed for AFB<sub>1</sub> by enzyme-linked immunosorbent assay. A reduction of 42.9% was achieved based on the median AFB<sub>1</sub> levels at baseline and after hand sorting (clean groundnuts), whereas an alternative estimate, based on the total AFB<sub>1</sub> in moldy and clean groundnuts, indicated a reduction of 96.7%, with a loss of only 2% of the groundnuts. By roasting the already clean sorted groundnuts, the AFB<sub>1</sub> reduction achieved (based on median levels) was 39.3%. This educational intervention on how to identify and remove moldy groundnuts was simple and effective in reducing AFB<sub>1</sub> contamination.

Key words: Aflatoxins; Groundnuts; Hand sorting; Intervention; Postharvest; West Africa

Aflatoxins are a group of naturally occurring contaminants produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus*. Because high temperature and high humidity promote fungal growth, aflatoxins are highly prevalent in staple foods, such as groundnuts and maize, grown in tropical regions. Of the four aflatoxins, B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most common type and has been classified as a group 1 human carcinogen (12). Aflatoxin exposure has previously been associated with immune suppression (13, 14, 28) and impaired child growth (8, 9) and may be lethal at acute high-dose exposure (24).

Aflatoxin contamination can occur in the field at harvest and during postharvest storage. Contamination levels are typically higher in the dry season after storage, when temperature and humidity are favorable for fungal growth and toxin production (22). Although aflatoxin contamination cannot be eliminated from food, it can be reduced to tolerable levels with rigorous aflatoxin reduction methods such as fertilization, irrigation, insect control, mechanized drying, and sorting throughout the food production chain.

In developed countries, aflatoxin exposure is low as a result of tight regulations and enforcement (2), but in developing countries, especially in rural subsistence farming

communities, some of the aforementioned mitigation strategies are not feasible (17). There is also a lack of regulation and enforcement (25), as well as low awareness of aflatoxin in the community (1, 15, 19, 31); hence, the risk of aflatoxin exposure is exacerbated. Furthermore, the effectiveness and long-term sustainability of aflatoxin reduction methods in developing countries are dependent on acceptability and implementation by the community that they are targeting. It is therefore fundamental that aflatoxin reduction methods are designed with local cultural practices taken into consideration.

Sorting is a simple and low-cost postharvest intervention method that involves the identification and then removal of discolored moldy food. It can be carried out manually by hand or electronically by color sorting machines. Previous research has indicated that both methods are effective in reducing aflatoxin contamination in groundnuts and maize (4–7, 16, 20, 21). Electronic color sorting, used within the commercial setting, is more suitable for developed regions that have the infrastructure to support it, whereas hand sorting is a more appropriate aflatoxin reduction strategy for rural subsistence farming communities, owing to its low cost and simplicity.

For some subsistence farming communities in sub-Saharan Africa, hand sorting to remove damaged and discolored food is local practice. The traditional sorting

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techniques used by these communities however are not likely to be effective for reducing aflatoxin contamination to sufficiently low levels, and this is evident by the high prevalence of aflatoxin exposure observed in sub-Saharan Africa (3, 26, 32). Hand sorting is typically undertaken by women within the community who are responsible for preparing and cooking family meals. Training these women to correctly identify and remove contaminated food would therefore be a beneficial aflatoxin reduction strategy for the entire community. The aim of this study was to evaluate the effectiveness of an educational intervention that involved training local Gambian women on how to identify and remove moldy groundnuts, a dietary staple in The Gambia, through hand sorting to reduce AFB<sub>1</sub> contamination. Previous research conducted in The Gambia has shown high levels of aflatoxin found in groundnut-based sauces (10), with exposure reflected by high levels of the aflatoxin–albumin adduct biomarker in blood samples collected from children (28).

## MATERIALS AND METHODS

**Participants and study setting.** In total, 25 women (aged between 20 and 40 years) were recruited in July 2014 from five villages located within the rural West Kiang region of The Gambia, ~100 km inland from the Atlantic coast. Participants were selected if they met the following criteria: have at least one child of weaning age, cultivate their own groundnuts at some time during the year, and are responsible for cooking family meals.

All participants provided informed consent. This study was approved by the Ethics Committee of the London School of Hygiene and Tropical Medicine, United Kingdom (LSHTM MSc Ethics Ref: 8083) and by the Gambian Government/Medical Research Council (MRC) joint ethics committee (SCC 1374v2).

**Questionnaire.** Before the educational intervention, each participant was provided with a questionnaire that gathered information about postharvest practices, groundnut consumption frequency, and aflatoxin awareness (11).

**Educational intervention and sample collection.** Participants were trained by a field assistant, in their local language, on how to identify and remove moldy groundnuts. The training session lasted 20 min and involved an explanation of the importance of hand sorting, its aims, and how it works. A practical demonstration of how to identify and remove the moldy nuts was provided by using photos of moldy groundnuts for illustration, followed by a question and answer session to address any queries. Visual aid reference sheets were given to the women, and they were also allowed to converse throughout the sorting process.

The study was conducted during the annual dry season (May to October) because this period is associated with higher levels of aflatoxin contamination. However, because this is the “hungry” season, home supplies of groundnuts were depleted. As such, after the training session, each participant was provided with ~5 kg of groundnuts taken from two large sacks (130 kg, with shells) that had been purchased from a local trader known to supply the local markets serving the villages included in the study.

After the training session, participants were instructed to remove the shells from the groundnuts. The shelled nuts were then pooled and mixed thoroughly by hand to ensure even distribution. Next, a 0.5-kg baseline sample per village was collected for the purpose of AFB<sub>1</sub> analysis. The groundnuts were subsequently

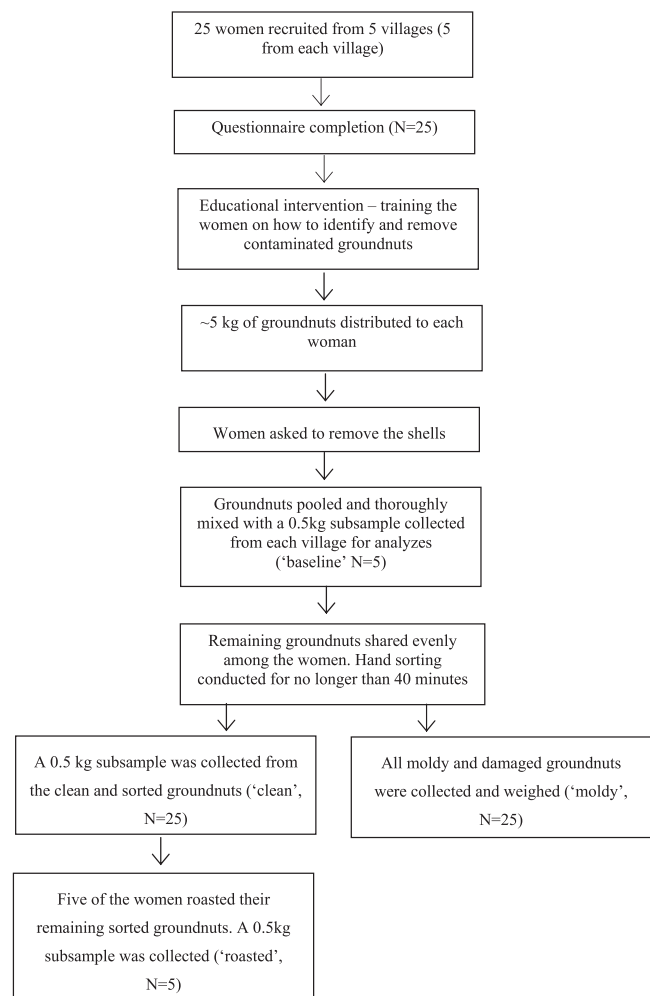


FIGURE 1. Flow chart showing the intervention procedure and groundnut sampling.

distributed evenly among the participants, who were instructed to sort their own portion of groundnuts, as previously taught, within a 40-min period. Participants were also asked to remove no more than 2% of the total weight. This process was completed on a village-by-village basis.

After sorting, the moldy groundnuts from each participant were weighed and collected ( $n = 25$ ). A 0.5-kg subsample of each of the 25 clean samples (see “Aflatoxin analysis”) was randomly collected for AFB<sub>1</sub> analysis. One participant per village was asked to roast 0.5 kg of the clean groundnuts, because roasting is a common practice for cooking. The cooking process involved placing the groundnuts in a metal cooking pot, with sand, over an open fire for ~15 min. The heated nuts were subsequently transferred to a blanket, wrapped up, and massaged to remove the skins. The nuts were then winnowed to remove the loosened skin. A 0.5-kg random sample from each batch of roasted groundnuts ( $n = 5$ ) was collected for AFB<sub>1</sub> analysis. Figure 1 illustrates the intervention procedure and the stages at which the groundnuts were sampled for AFB<sub>1</sub> analysis.

**Aflatoxin analysis.** Of the groundnut samples collected at baseline (“baseline,”  $n = 5$ ), after hand sorting (“clean,”  $n = 25$ , and “moldy,”  $n = 25$ ), and after roasting (“roasted,”  $n = 5$ ), 100 g of each sample was transported to Queen’s University Belfast for AFB<sub>1</sub> analysis. A blind analysis was performed.

TABLE 1. *AFB<sub>1</sub> concentrations in groundnut samples before and after the training intervention*

Groundnut sample	<i>n</i>	AFB <sub>1</sub> (μg/kg) <sup>a</sup>
Baseline	5	0.49 (0.27–0.52)
Moldy sorted	25	141.38 (8.22–813.86)
Clean sorted	25	0.28 (0.24–0.54)
Roasted	5	0.17 (0.14–0.27)

<sup>a</sup> Values are median (interquartile range).

Sample preparation was modified based on the protocol of the AFB<sub>1</sub> enzyme-linked immunosorbent assay (ELISA) kit (Euro-Proxima B.V., Arnhem, The Netherlands). Groundnut samples were ground into a homogenous powder with a laboratory blender (Christison Particle Technologies, Newcastle upon Tyne, UK). Sample powders (3 g) were homogenized in 9 ml of 80% methanol, mixed, and then centrifuged at 2,000 × *g* for 10 min. The supernatant (250 μl) was further diluted with 750 μl of dilution buffer (from the ELISA kit) and purified by 1 ml of *n*-hexane. The ELISA analysis was conducted following the protocol of the AFB<sub>1</sub> ELISA kit (EuroProxima B.V.) as previously described by Pourelmi et al. (23). A negative (clean sample) and a positive sample (clean sample spiked with 1 ng/g AFB<sub>1</sub>) were used as controls for each run. The quantification range of the AFB<sub>1</sub> ELISA kit was 0.04 to 2.4 μg/kg. Where necessary, a further dilution was applied. The limit of detection was 0.04 μg/kg, with a coefficient of variation of 7 to 11%.

**Data analysis.** AFB<sub>1</sub> results are expressed as median and interquartile range, because variables did not satisfy normality criteria. AFB<sub>1</sub> reductions are presented as percentages. Questionnaire results are presented as frequencies, percentages, or both.

## RESULTS

**Postharvest practices (storage and preparation).** All 25 women responded to the survey. Seventeen (68%) of the 25 women reported purchasing groundnuts from their local market. Most of the women reported using sealed plastic containers (40%) or sacks (44%) to store their groundnuts, or they used a combination of these two materials (16%). All women reported removing moldy groundnuts before consumption, of which 40% threw them away in their backyards and 8% fed them to animals. Sixty-four percent reported washing the groundnuts before consumption. Reasons for washing included removing residual powder (44%), removing farming chemicals (8%), and removing dirt (8%).

**Groundnut consumption frequency and other dietary responses.** All of the women reported having a child <24 months of age. The majority (88%) of these children were consuming breast milk along with weaning foods. For the frequency of consumption, groundnuts were reported as the most common weaning food ingredient (68%). Other

foods included corn porridge (8%), a mixture of groundnuts and rice (4%), and animal milk (4%). Fifty-six percent of the women reported that they, and 60% of their children, consumed groundnuts in the last week three or more times. Other foods consumed during the rainy season included oily food (88%), green leafy food (84%), rice (76%), and soup (52%).

**Aflatoxin awareness.** Twenty-two (88%) of 25 women responded that they had heard of aflatoxins and were aware of the “toxic” effect. Health workers (77%) followed by friends (23%) were the main sources of this information. Twenty (91%) of 22 women thought aflatoxin exposure was associated with “heart disease,” which is a common local way of describing liver disease and cancer; one woman mentioned that it was associated with liver damage and another stated that it was associated with disease in general, but she did not specify what disease.

### AFB<sub>1</sub> concentrations before and after hand sorting.

Table 1 summarizes the AFB<sub>1</sub> concentrations before and after the intervention. AFB<sub>1</sub> concentrations of the five baseline samples were 0.26, 0.27, 0.49, 0.52, and 560.97 μg/kg. The moldy groundnuts had AFB<sub>1</sub> levels ~500-fold higher than the clean sorted samples and ~800-fold higher than the roasted samples.

**Intervention effectiveness.** Based on the median AFB<sub>1</sub> concentrations in the baseline and clean samples (Table 1), the AFB<sub>1</sub> reduction achieved by hand sorting was 42.9%. By roasting the already clean sorted groundnuts, the AFB<sub>1</sub> reduction achieved was 39.3%.

Table 2 shows an alternative and potentially more accurate calculation of AFB<sub>1</sub> reduction, based on measured AFB<sub>1</sub> levels after hand sorting into moldy groundnuts and clean groundnuts. In total, 1,533 g of moldy groundnuts (∑ weight of the 25 sorted moldy groundnuts) was removed from an initial 79,388 g of shelled groundnuts (at baseline), equating to a 2% loss of food. The total amount of AFB<sub>1</sub> found in the moldy groundnuts was 905.5 μg (∑ weight of each moldy groundnut sample × AFB<sub>1</sub> concentrations). The total amount of AFB<sub>1</sub> found in the clean sorted groundnuts was 29.9 μg (∑ weight of each clean groundnut sample × AFB<sub>1</sub> concentrations). Based on these values, a reduction of 96.7% in AFB<sub>1</sub> was achieved.

## DISCUSSION

**Hand sorting.** This study evaluated the effectiveness of an educational intervention that involved training local Gambian women on how to identify and remove moldy groundnuts, to help reduce the level of aflatoxin contamination in their diets. Comparing the median values at

TABLE 2. *Calculation of AFB<sub>1</sub> reduction based on measured AFB<sub>1</sub> levels after sorting into moldy and clean groundnuts*

Wt of baseline shelled nuts (g)	Wt of moldy nuts removed (g)	AFB <sub>1</sub> amount in moldy nuts (μg)	AFB <sub>1</sub> in remaining clean nuts (μg)	Reduction in AFB <sub>1</sub> (%)
79,388	1,533	905.5	29.9	96.7

baseline and in clean sorted groundnuts, these women achieved a 42.9% reduction in AFB<sub>1</sub> concentrations. It is important to consider that this level of reduction is a conservative estimate based on inaccurate sampling at baseline. For example, the amount of AFB<sub>1</sub> present in the moldy groundnuts that was removed by hand sorting contained far higher levels than calculated from the median AFB<sub>1</sub> levels obtained from the five baseline samples. Furthermore, the 0.49 µg/kg median AFB<sub>1</sub> concentration in the baseline samples seems to be far below the 10-µg/kg African regulation level (30). By back calculating the baseline AFB<sub>1</sub> concentrations based on merely the amount present in the moldy groundnuts (where all AFB<sub>1</sub> was measured), the AFB<sub>1</sub> concentration at baseline would be 11.4 µg/kg, a value that is above the African regulation level. The latter calculation, deemed a more accurate estimate of AFB<sub>1</sub> at baseline, leads to the estimate that 96.7% of aflatoxin was removed by sorting. This value may be an overestimate, because it is also based on random sampling of the clean groundnuts to estimate the remaining aflatoxin contamination of these groundnuts, but it is likely to be more accurate because the original baseline calculation is distorted by the heterogeneous distribution of aflatoxin.

Regardless of the method used to calculate the reduction rate in the current study, it is evident that hand sorting can reduce aflatoxin contamination in groundnuts to a certain extent when adequate training is provided. There is a paucity of studies in the literature that have evaluated hand sorting as a stand-alone aflatoxin reduction strategy for groundnuts, performed at a subsistence farming level after adequate training. One study has evaluated the effectiveness of a package of postharvest intervention measures, including hand sorting along with adequate drying, storage, and use of insecticide, at subsistence farms in Guinea (29). A 69% reduction in AFB<sub>1</sub> in groundnuts was achieved; however, it is difficult to establish how much of this reduction was attributable to hand sorting.

Some of the previous studies that did evaluate the effects of hand sorting as a stand-alone method were conducted within a commercial setting, comparing manual hand sorting with electronic color sorting (4) and fluorescence color sorting (21). Hand color sorting seems the most effective method for removing aflatoxin contamination in groundnuts, followed by machine color sorting (~16 to 70% of aflatoxin can be removed), whereas fluorescence sorting (37.2% of aflatoxin were removed) seems to be the least effective method (4, 21). Hand sorting, however, is a time- and labor-consuming process that is not ideal for large food manufacturers in developed regions. Conversely, owing to its simplicity and low cost, hand sorting is more suitable for subsistence farming communities in developing countries.

Hand sorting is already a local practice in some subsistence farming communities. In fact, all the women in the current study reported sorting their groundnuts before consumption. However, the frequent aflatoxin exposure reported in the region (3, 27, 28) suggests the hand sorting method used may not be effective in reducing aflatoxin contamination. This high exposure is likely because of the many variations of the hand sorting method used by these

women; thus, they are not reaping the maximal benefit shown to be possible through the use of a specific educational protocol, as demonstrated by this intervention study.

**Roasting.** Roasting groundnuts is considered another aflatoxin reduction strategy that is a local practice in some subsistence farming communities. It has been reported that dry roasting groundnuts can reduce aflatoxin concentrations by 45 to 83% (18). Yazdanpanah et al. (33) found in a series of experiments that roasting nuts at 150°C for 30 min can significantly reduce AFB<sub>1</sub> and AFB<sub>2</sub> concentrations without compromising the taste of the nuts. In the current study, the clean sorted groundnuts were roasted for 15 min, and a further reduction (39.3% based on median levels of clean sorted versus roasted nuts) of AFB<sub>1</sub> was achieved. However, this finding should be viewed with caution, mainly because the temperature throughout roasting was not recorded; hence, it is unknown whether the roasted groundnuts were palatable. In addition, only five samples (one sample per village) of the clean sorted groundnuts were roasted, making it difficult to establish whether the roasting method described in the current study is a valuable aflatoxin reduction method in its own right.

**Aflatoxin awareness.** Most of the women (88%) in the current study reported that they had heard of aflatoxins and were aware of their associated health risks. The literature suggests that aflatoxin awareness is generally low in sub-Saharan Africa, especially among those in subsistence farming communities. For example, Jolly et al. (15) conducted a cross-sectional survey to determine factors that are associated with high AFB<sub>1</sub> levels in the Ashanti region of Ghana, where the individuals are mostly subsistence farmers. They found that the majority of participants surveyed (135 of 142, 92.3%) reported having no knowledge of aflatoxin. It is important to consider, however, that the Jolly et al. study (15) was conducted 10 years ago, and perhaps more individuals are now aware of aflatoxins. Nevertheless, most of the women in the current study had heard of aflatoxins and 56% reported that they, and 60% of their children, had consumed groundnuts in the last week three or more times.

**Strengths and limitations of the study.** In contrast to other published studies, this study focused on the effectiveness of hand sorting as a stand-alone aflatoxin reduction method for groundnuts performed at the subsistence farming community level, thus offering valuable information for public health-targeted interventions. Conversely, there are some limitations of this study such as the small sample sizes, with only five baseline samples collected that had large variations in AFB<sub>1</sub> concentrations. The baseline calculation is therefore distorted by the heterogeneous distribution of aflatoxins, making it difficult to determine the true impact of hand sorting. A larger sample size along with an improved sampling method would help achieve a more representative sample of aflatoxin contamination from the onset and help determine the true impact of hand sorting and roasting

carried out by these rural Gambian women. Furthermore, market-purchased groundnuts were used that potentially could have lower levels of aflatoxin contamination than home-cultivated groundnuts, prompting further study to be conducted during and shortly after groundnut harvest season when home-grown groundnuts are available.

This study evaluated the effect of an educational intervention that involved training local Gambian women on how to correctly identify and remove moldy groundnuts by hand sorting, a traditional postharvest method typically used in some subsistence farming communities. Significant reductions in AFB<sub>1</sub> were apparent when the amounts of AFB<sub>1</sub> in the sorted clean and moldy groundnuts were compared. Subsequent roasting of the clean sorted groundnuts resulted in further reductions. The study was compromised by small samples sizes at baseline and the use of market-purchased groundnuts, which are not directly associated with intake from home-cultivated groundnuts. Further research taking these issues into consideration is warranted to determine the true impact of this educational intervention.

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