



Moisture and Aflatoxin Contents of Kenyan Market Peanuts and Decontamination with Water, Lime and Ultraviolet Radiation Treatments

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Authors' contributions

This work was carried out in collaboration among all authors. Author JWW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JKI and LGN managed in study development and edited the manuscript. Author JWW managed the submission of the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In the recent past, aflatoxin in peanuts and products has been a subject of controversy in Kenya. In the early 2019, some brands of locally manufactured peanut butter were withdrawn from the market shelves on account of containing aflatoxin higher than the national tolerance. It has been established that Kenyan market peanuts contain high levels of aflatoxin. Roasting is reported to have little effect on the aflatoxin content. This study was designed to assess the effect of specific treatments prior to roasting in reducing the aflatoxin levels to below the tolerance. This would then ensure compliance of the toxin levels in the roasted peanuts and the products with the national tolerance. The treatments included soaking in water, in lime, and UV irradiation. The peanut samples were collected from 20 vendors in the Main Cereal Market in Nairobi and brought to the laboratory of the Kenya Bureau of Standards (KEBS). They were analysed for moisture and aflatoxin content. Each sample was subjected to the treatment and reduction was evaluated in aflatoxin to the tolerance. The moisture content of the peanuts varied from 5.2 – 8.4% with mean of 6.5%. Moisture almost complied with the optimum for storage of 8.0%. The total aflatoxin contents

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varied from 3.3 – 38.5 ppb with mean of 14.8 ppb. Up to 45% samples had aflatoxin above tolerance of 10 ppb.

There was positive and significant ($p < 0.01$) correlation between aflatoxin levels and moisture content. Treatment of the peanuts with water (cold & warm) and warm lime and irradiation with UV all managed to reduce the aflatoxin contents to below the tolerance, in the order lime>warm water>cold water>UV irradiation. The study concluded that the moisture content of the market peanuts in Kenya, almost complies with recommended optimum for storage the tolerance, but the mean aflatoxin content was well above the tolerance. However, the aflatoxin levels can be lowered effectively by soaking in water, lime, and by irradiation with UV.

Keywords: Peanuts; Kenya; moisture; aflatoxin; decontamination; water; lime; UV.

1. INTRODUCTION

Early in 2019, Kenya Bureau of Standards (KEBS) recommended withdrawal of some brands of locally manufactured peanut butter from the market because they contained aflatoxin levels higher than the national tolerance of 10 ppb. The problem was traced to the raw peanuts which contained such high levels of aflatoxin that even after roasting and shelling, the levels of the toxin in the peanut butter produced there from was still higher than the tolerance. The other forms in which peanuts are consumed in Kenya include the roasted with shell in various variants, the roasted and shelled and as flour from raw peanuts in a mixture with cereal flours for use in porridge preparation. These products obviously contain higher levels of the toxin than the tolerance [1]. It is shown that roasting does not reduce aflatoxin levels in peanuts substantially [2].

The nuts shelled with machine also does not lower the toxin to below the tolerance due to the intermixing of the shell and kernel during shelling so that the former contaminates the latter. Reports indicate that many of the peanuts and peanut products analyzed in Kenyan markets do not meet the standards in terms of Aflatoxin levels [3]. Specific levels of aflatoxin in peanuts from Kenya have been reported to be from 0 to 7525 ppb [4].

Levels of up to 513 ppb of other peanut products were also reported during a study conducted in Taiwan [5]. The reports from KEBS also, indicate that the peanuts products produced by the locally manufactured in Kenya mainly the peanut butter is heavily contaminated with the toxin [6]. Roasting, however, either oven or microwave has been reported not to lower the aflatoxin content significantly [7]. During machine shelling, some aflatoxin is likely to be transferred from the shells to the cotyledons thereby being

transmitted to the peanut butter. This way also the market roasted and/or cleaned peanuts will have aflatoxin levels possibly higher than the tolerance of 10 ppb.

The most practical solution to this problem is reduction of the aflatoxin in raw peanuts to the tolerance or below prior to roasting. Cooking in alkali (Nixtamalization) has been found to reduce levels of aflatoxin in maize substantially [8]. Peanuts and products are widely consumed in Kenya, but mainly as roasted peanuts and peanut butter. These products have been reported to have higher residual aflatoxin than the tolerance and therefore expose the consumer to Aflatoxicosis. The peanuts for the study were procured from vendors in the main Cereal Market in Nairobi which is Nyamakima market. Most of them are imported from Malawi. According to the work done in 2013, the peanuts contain higher levels of aflatoxin than the peanuts from other markets in the Country [9]. This study was designed to evaluate the effectiveness of specific treatments which include soaking in water, soaking in lime (calcium hydroxide solution) and irradiation with UV to lower the total aflatoxin levels to below the tolerance of 10ppb prior to roasting.

2. STUDY METHODOLOGY

2.1 Study Design

The study was cross-sectional in design with analytical component. Samples of peanuts were randomly collected from 20 vendors in Nyamakima. These were analysed for moisture and aflatoxin content. Then batches from each sample were soak-washed in cold tap water, warm water and lime, then irradiated with UV. For each treatment, the peanuts were evaluated for their reduction in aflatoxin with reference to the national tolerance for total aflatoxin.

2.2 Methods

2.2.1 Source of the peanuts for the study

The peanuts for the study were obtained from Nyamakima, the main Cereal Market in the Nairobi Metropolitan. The Market is located in the Central Business District of the Nairobi Metropolitan. It is the largest market for cereal grains, legumes, pulses and nuts with individual vendors practicing wholesale and retail selling. The market is located as pinned and shown by the arrow in the Road map of Nairobi Metropolitan in Fig. 1.

2.2.2 Sampling of the peanuts for analysis and treatments

The sampling frame consisted of those vendors who sold peanuts only. They were 40 in total. They were all arranged in a row. A sample of 3 kg was taken from each of every other vendor, totalling 20 samples. The samples were then brought to the Laboratories of the Kenya Bureau

of Standards (KEBS) and stored in a cool dry place to await analyses and the pre-treatments.

2.2.3 Pre-treatments of raw peanuts

The following pre-treatments were applied to the raw peanuts to reduce the levels of aflatoxin before roasting to below the tolerance for total aflatoxin of 10 ppb.

2.2.4 Treatment with cold water

This treatment was carried out with cold tap water and warm water at 50 – 60°C. The peanuts were passed through a 4 mm sieve to remove the small particles and debris. Then about 100 g were accurately weighed and placed in a 1000 ml beaker. Then, 250 ml of cold tap water was added and the peanuts were gently agitated with glass rod for the time 2, 4, 6, and 8 minutes respectively. The water was decanted and the peanuts were dried of the surface water using paper towels. Then, 50 g of peanuts were placed in a mortar and ground with a pestle to a fairly fine powder. Of the powder, about 20 g were weighed accurately and analysed for aflatoxin.

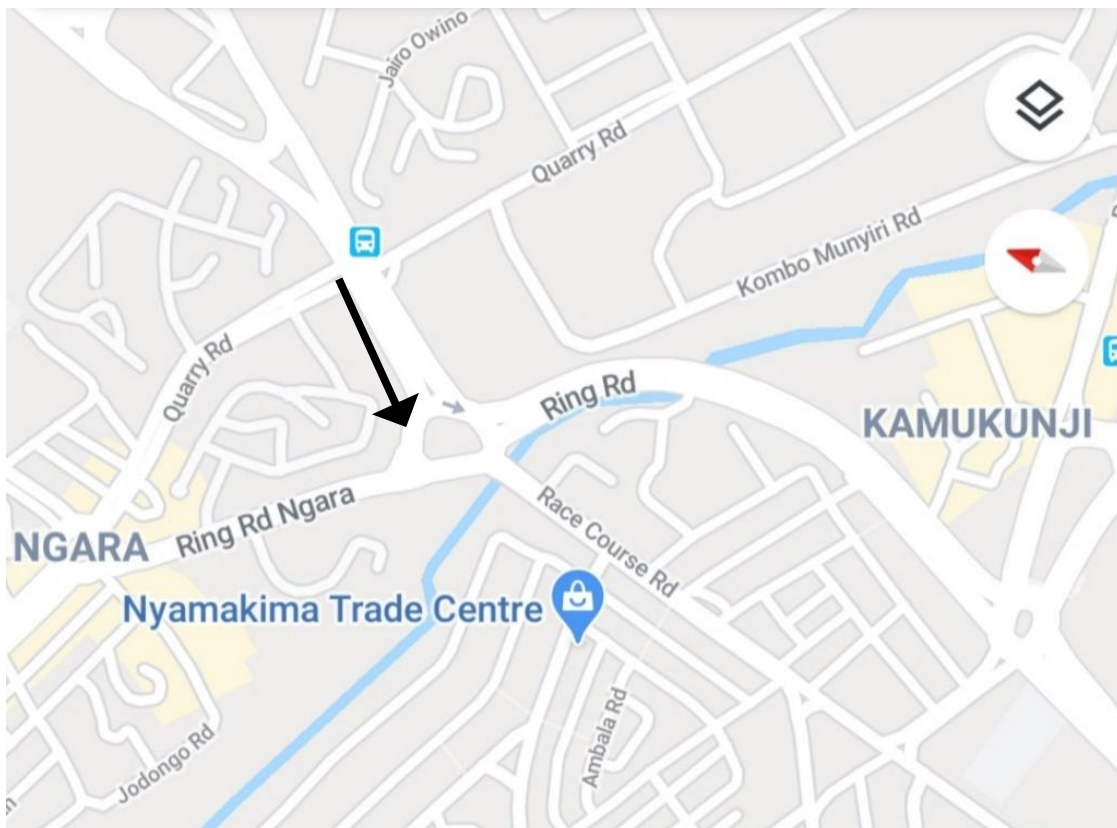


Fig. 1. Road map of nairobi metropolis showing the position of nyamakima market

2.2.5 Treatment with warm water

This treatment was carried out in exactly the same way except that the cold water was warmed to 50 – 60°C.

2.2.6 Treatment with lime (calcium hydroxide)

Lime (calcium hydroxide) was prepared in water at concentrations of 0.01, 0.02, 0.03 and 0.04% per 100 ml water. Each time, the solution was warmed to 50–60°C and used for treatment of the peanuts just like with warm water. Treatment time varied between 1–4 minutes at intervals of 1 minute.

2.2.7 Treatment with UV radiation

The peanuts of 500 g free from debris were weighed and evenly spread on a black polythene paper.

A UV irradiation of wavelength 346 nm was directed from the Ultra-violet lamp source placed at 30 cm above the peanuts for up to 6 hours. Then, samples of 50 g of the peanuts were taken each hour for analysis of aflatoxin content.

2.3 Analytical Methods

2.3.1 Determination of moisture

Moisture was determined in the peanut samples by AOAC methods [10] as follows:

The peanuts of about 20 g free from dirt and debris were crushed and ground in a mortar and pestle to a fine powder. Moisture content was determined using a thermostatically controlled air-oven at temperature of 105°C. The clean aluminium moisture dishes were conditioned by drying in an oven for about 10 minutes, cooling in a desiccator to room temperature and weighing. About 5 g peanut powder was accurately weighed onto it.

The dish and contents were transferred to the oven and dried to constant weight then cooled to room temperature in a desiccator and weighed. Moisture content was calculated as percent weight loss as follows.

$$\text{Moisture (\%)} = (W1-W2)/W1*100$$

Where,

W1=Weight (g) of the sample before drying
W2= Weight (g) of the sample after drying

2.3.2 Analysis of aflatoxin

Enzyme Linked Immunosorbent Assays (ELISA) method was used to analyse the total aflatoxin explained by Helica Biosystems International, as used in a study that was done in Kisumu Kenya [11] as follows:

2.3.2.1 Sample preparation and extraction

About 50 g of peanuts free from dust and debris were weighed and crushed using motor and pestle to a fine powder. Then about 20 g were weighed accurately into a flat bottomed flask and 100 ml of 70% methanol added to extract the aflatoxin. The flask contents were mixed in a laboratory electric shaker for 30 minutes. The mixture was allowed to settle then filtered through a Whatman filter paper Number 540. The filtrate was used for analysis of aflatoxin content.

2.3.2.2 Analysis

The reagents to be used were brought to room temperature from the preserving temperature of 2 to 8°C Each sample and the standard to be tested was assigned to a single dilution micro well and set on to the holder. Equal number of the antibody coated micro titre wells were placed on another holder. 200 µL of the Aflatoxin-HRP Conjugate solution (composed of conjugated peroxidase in buffer with preservatives) was dispensed in to each mixing well. By the use of a new pipette tip, 100 µL of each sample and the standard was added to each appropriate mixing well that has the conjugate.

By the use of the pipette and the tip, the solution was mixed thoroughly for at least 3 times and then a 100 µL from each mixing well was transferred to the corresponding Antibody coated Micro-titre well, then incubated for 15 minutes at room temperature.

Afterwards, the content was decanted into the wash sink and each micro well was thoroughly cleaned by filling each PBS-Tween wash buffer solution of about 6.8-7.0 pH, then decanting the buffer in to the wash sink. The washing was repeated for 5 times. The micro wells were tapped facing down on a layer of an absorbent towel to remove the residual buffer. 100 µL of the substrate solution (composed of stabilised tetramethylbenzidine) was added into each micro well by the use of the pipette and was incubated

for 5 minutes while covering with an aluminium foil to protect from the direct light. 100 μ L of the stop solution (Acidic solution) was added into each well in the same sequence and as the substrate solution was added. The optical density (OD) was obtained from each well with a microtiter plate reader using 450 nm wavelength filter and the OD for each micro well was recorded. A dose-response curve was constructed using the OD values which were expressed as percentage (%B/Bo) of the OD of the zero (0.0) against the Aflatoxin content of the standard. Unknown were measured by interpolation from the standard curve. The ratio of dilution of the samples was 5:1 in 70% Methanol hence the level of Aflatoxin shown by the standards were multiplied by 5 in order to indicate the Aflatoxin in ppb.

2.4 Statistical Analysis of Data

Linear regression analysis was used to establish the predictor effect of moisture content on the aflatoxin content of groundnuts. Significance was tested at $P < .05$. The laboratory data was analysed using R Statistical Package [12]. Descriptive statistics of aflatoxin levels in the treated groundnuts were generated. Analysis of variance (ANOVA) was used to establish significant differences at $P < .01$ in the treatments applied that had no quantitative measures in representation. The different means were separated using Tukey's HSD test [13].

For treatments that were in quantitative measures including time of UV exposure, predictor effect on aflatoxin content was established using linear regression test. Significance was tested at $P < .05$.

3. RESULTS AND DISCUSSION

3.1 Moisture and Aflatoxin Contents of the Peanuts

The moisture and aflatoxin contents of the peanuts are shown in Table 1. The moisture contents of peanuts varied from 5.2 – 8.4%, with mean of 6.5%. The moisture content was significantly different from each other at $P < .05$. As per East African Standard (EAS 57:2006), the optimum moisture content for storage of peanuts is 8.0% to stop the mouldy growth including the Mycotoxigenic molds. Against this level, only 5 (25%) samples were slightly above the tolerance moisture content (8.0 – 8.4%).

These values were, however, not significantly different from each other at $P < .05$. During storage for prolonged periods, it is possible that these 5 samples could easily effectively set up water activity levels that would encourage growth of aflatoxin producing molds if present. It is a requirement in Kenya that all market peanuts be dried to meet the legal requirements with regard to moisture content. Possibly most or all the peanuts had therefore been dried by the vendors prior to bringing to the market. It is most likely that the peanuts purchased from the farmers contained moisture well above the tolerance limit.

This is a problem for those processors who purchase the peanuts directly from farmers and store for long periods of time to await processing.

The extensive molds growth not only produces aflatoxin but may affect extensive rotting. The peanuts in the farmer's stores may also accumulate aflatoxin due to the favourable conditions for growth of the Mycotoxigenic molds. The aflatoxin contents in the present study varied from 1.6 – 38.5 ppb with mean of 14.8 ppb. The aflatoxin contents were significantly different from each other at $P < .01$. The tolerance for total aflatoxin content in peanuts in Kenya is 10 ppb.

Based on this, the results indicate that 9 samples (representing 45%) had aflatoxin levels higher than the tolerance (17.1 – 38.5%). The mean aflatoxin content of the samples was also higher than the tolerance. The highest level of aflatoxin found in this study is, however, much lower than the level reported in market peanuts of up to 2377.1 ppb [9] which is about 50-times higher than the levels obtained in this study. Those peanuts with aflatoxin contents higher than the tolerance when roasted, shelled and processed into peanut butter may yield products with aflatoxin contents higher than the tolerance.

There was a positive significant correlation ($P < .01$, $R^2 = 0.5035$) between moisture and aflatoxin contents. A positive correlation between moisture content and the aflatoxin concentration in fragrans seeds (nutmeg) and chilli was also reported [14].

3.2 Effect of Pre-treatments on the Aflatoxin Contents of the Peanuts

Four different treatments were evaluated for their effectiveness to lower the aflatoxin contents of raw peanuts as under:

Table 1. Moisture and aflatoxin contents of market peanut by vendors

Sample	Moisture Content (%)*	Aflatoxin content (ppb)*
1.00	5.5±0.1 ^a	4.9±0.0 ^c
2.00	5.2±0.1 ^a	7.2±0.7 ^d
3.00	7.2±0.4 ^{cd}	29.7±0.0 ^h
4.00	5.7±0.1 ^{ab}	3.2±0.0 ^b
5.00	5.4±0.1 ^a	2.2±0.0 ^a
6.00	5.8±0.0 ^{ab}	3.7±0.0 ^b
7.00	5.5±0.1 ^a	3.2±0.00 ^b
8.00	7.6±0.1 ^{de}	17.1±0.0 ^f
9.00	8.0±0.2 ^{de}	38.5±0.1 ^l
10.00	8.0±0.2 ^{de}	15.6±0.0 ^e
11.00	5.9±0.8 ^{ab}	7.2±0.0 ^d
12.00	6.2±0.0 ^{ab}	19.7±0.0 ^g
13.00	8.4±0.2 ^e	35.5±0.0 ^k
14.00	8.1±0.0 ^{de}	32.5±0.0 ^j
15.00	5.2±0.2 ^a	31.7±0.0 ⁱ
16.00	6.5±0.2 ^{bc}	5.3±0.0 ^c
17.00	5.7±0.3 ^{ab}	5.1±0.2 ^c
18.00	5.7±0.0 ^{ab}	3.3±0.2 ^b
19.00	8.3±0.0 ^e	29.2±0.0
20.00	5.9±0.1 ^{ab}	1.6±0.0 ^a
Mean	6.5±1.1	14.8±13.0

*Mean ±SD (N = 20). Means down a column having the same superscript are not significantly different from each other at P ≤ .05.

3.2.1 Effect of treatment with cold and warm water

This treatment was carried out with cold tap water and warm water at 50 – 60°C for 2, 4, 6, and 8 minutes and the results are shown in Table 2. The aflatoxin levels of the peanuts dropped steadily after treatment with both cold and warm water. After treatment with cold water for 4 minutes, the aflatoxin level fell slightly below the tolerance of 10 ppb (by 32.2% from initial level).

After the same treatment time with warm water, the aflatoxin level dropped significantly to less than the tolerance (by 58.9% from the original level).

The levels of aflatoxin in the peanuts after treatment with cold and warm water were significantly different (P < .01) from each other.

After 4 minutes, the treatments steadily decreased the aflatoxin levels of the peanuts, but more rapidly with the warm than cold water.

To attain a high degree of safety therefore, it would be recommended that the peanuts be treated with warm water for 4 minutes and with cold water for 5 minutes to ensure lowering of the aflatoxin levels to below the tolerance. This treatment is just a washing process.

The solvent work better while higher than the lower temperatures. It was noticed that beyond 60°C, the peanuts tended to show signs of cooking and oxidation.

Besides dislodging of the aflatoxin, the peanuts were cleaned of dirt and the microbial loads were reduced to render the product healthier and more wholesome.

Table 2. Effect of treatment with water on the aflatoxin contents (ppb)* of the peanuts

Time (Minutes)	Cold water	Warm (50-60°C)
0	15.56±0.02 ^{Ca}	15.56±0.02 ^{Ca}
2	11.68±0.00 ^{Cd}	10.52±0.00 ^{Cd}
4	9.77±0.00 ^{Cc}	6.40±0.01 ^{Cd}
6	6.30±0.01 ^{Ce}	2.67±0.00 ^{Cg}
8	4.31±0.00 ^{Cf}	Nd

*Mean ±SD (N = 10). Means with a similar uppercase letters followed by a different lowercase letter in the superscript are statistically different at P < .001. nd-not detected

3.2.2 Treatment with lime

Lime was used at concentrations of 0.01, 0.02, 0.03 and 0.04% in water for 1 – 4 minutes and data collected are shown in Table 3.

With increase in the concentration of the lime, there was significant ($P < .01$) reduction in the level of aflatoxin content. The maximum reduction in aflatoxin level of peanuts was realized at a lime concentration of 0.04% after four minutes of treatment (98.2%). The time of soaking in lime did not significantly ($P < .01$) influence the level of aflatoxin reduction. The treatment time required to reduce the aflatoxin to the tolerance decreased significantly with increase in the concentration of lime.

The time of soaking in lime required to reduce the concentration of aflatoxin peanuts to less than the tolerance of 10ppb decreased with increase in concentration of lime. This time was 3 minutes at 0.01% lime concentration but, it was reduced to only 1 minute at 0.02 – 0.04%. The recommendation is therefore that treatment for these time lime combinations would be adequate to reduce the aflatoxin level to way below the tolerance of 10 ppb pre-roasting. The higher concentrations of lime should be preferred because the treatment would be able to add significant amounts of calcium, a crucial nutrient in the diet. All the roasted variants of peanuts and the products like peanut butter would therefore contain less than the tolerance of aflatoxin.

The clearance of the aflatoxin with the lime was more efficient than with the warm water. The rate of removal increased with the concentration of the lime and the time of treatment.

This is probably attributable to the fact that water merely washes the toxin from the surface of the peanuts, additionally some aflatoxin is destroyed by the alkali. Effective reduction of aflatoxin by the use of lime was also reported [8].

3.3 UV Irradiation

Continuous exposure of the peanuts to UV irradiation for 2, 4 and 6 hours yielded the results shown in Fig. 2. The exposure significantly ($P < .01$) reduced the aflatoxin content of the peanuts. A change in time by one hour accounted for 64.6% reduction in the aflatoxin levels. A study was also done and it also showed that aflatoxin in peanuts was reduced as time increased [15]. UV wavelength of 346 nm was used and the regression line represented by $R^2 = 0.646$, $P = .01$, at 95% confidence interval, on the linear regression line ($y = 12.2 - 2.1x$), $y = 10$ the tolerance for total aflatoxin was attained at the time $x = 1.0$. The level of aflatoxin fell below the tolerance after treatment for slightly less than two hours.

A study was carried out using peanuts which were spiked with aflatoxin to vary the concentration. The study showed a similar effect to the results of this study. The UV irradiation to the known aflatoxin concentration spiked in water but the UV irradiation doses also varied [16].

Only one band of UV irradiation was available for use. The trial required treatment at different wavelengths and times of exposure to help optimize them to reduce the levels of aflatoxin to well below the tolerance. The use of UV irradiation to inactivate the aflatoxin, has been proven to be effective, however the degradation products of aflatoxins and their safety or toxicity has not been clear [17].

Use of UV irradiation in treatment of storage cereals, was found to be the most effective method to reduce levels of aflatoxin in grains during storage from deterioration, hence providing safe food and also minimising the loss [18]. In this study, only a monolayer packing was applied.

Table 3. Effect of treatment with lime solution on aflatoxin contents (ppb)* of the peanuts

Lime Conc. (percent)/Treatment time (Minutes)	1	2	3	4
Initial content of aflatoxin. (ppb)	24.0±16.1 ^{Ca}	24.0±16.1 ^{Ca}	24.0±16.1 ^{Ca}	24.0±16.1 ^{Ca}
0.01	13.30±13.3 ^{Ca}	11.6±11.6 ^{Ca}	3.2±1.1 ^{Ca}	2.4±1.3 ^{Ca}
0.02	5.73±0.16 ^{Ca}	2.40±2.5 ^{Ca}	1.36±0.3 ^{Ca}	1.1±0.0 ^{Ca}
0.03	3.63±4.2 ^{Ca}	0.8±0.1 ^{Ca}	0.84±0.42 ^{Ca}	Nd
0.04	Nd	0.4±0.5 ^{Ca}	0.5±0.04 ^{Ca}	Nd

*Mean±SD (N = 3) Values with a similar uppercase letters followed by a different lowercase letter in the superscript are statistically different at $p < .01$. Nd = Not detected

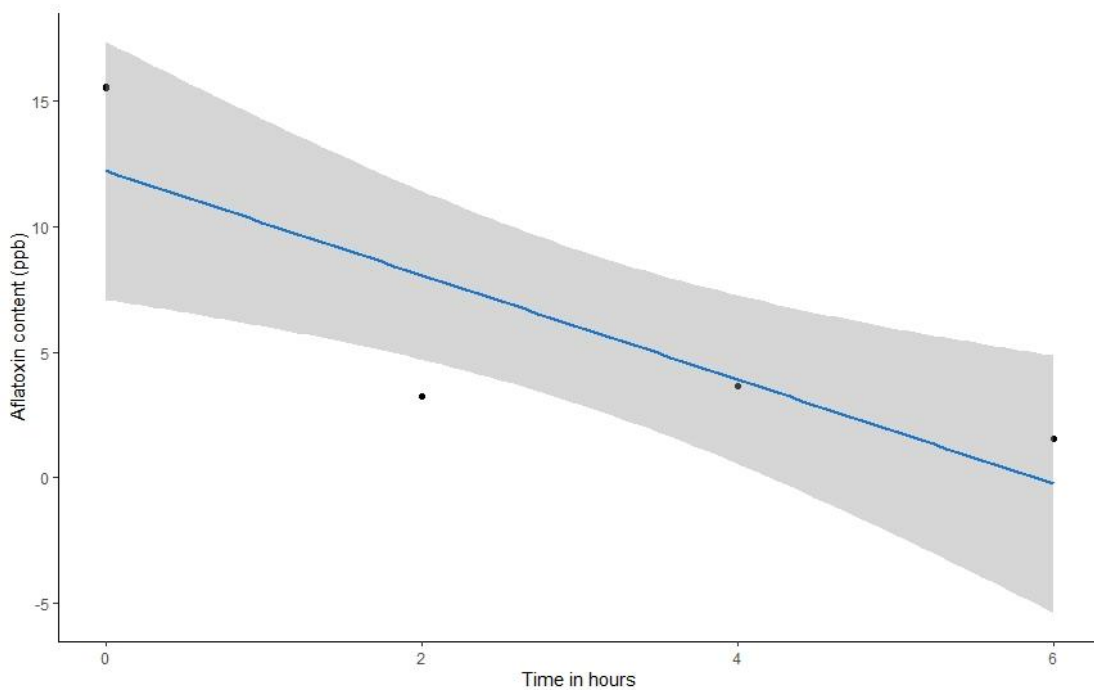


Fig. 2. Effect of UV irradiation on the aflatoxin content of peanuts

For an effective reduction of Aflatoxin while using the UV irradiation, a thin layer spread of the cereals has shown to be impactful, where the decrease of the Aflatoxin levels correlated strongly with the thickness of the sample layer [19]. In a practical commercial situation, more than a monolayer packing will be used. This might lead to an increase in the time for irradiation treatment to reduce the levels to below the tolerance, although this could possibly be counterbalanced by increasing the UV energy to optimum.

3.4 Discussion

The aflatoxin levels established in this study were fairly moderate, probably because they had been dried and almost complied with the optimum levels for storage, and therefore even in storage either in the farmer's fields or in the market stores, the Mycotoxigenic moulds would hardly grow to produce the toxin. Aflatoxin levels of up to 2000ppb have been reported in raw peanut flour from the Micro-Small-Medium Enterprises (MSME) [20]. The flour was meant for blending with flours of cereals, legumes and starchy roots, as porridge for vulnerable groups, some of which had average of up to 56ppb total aflatoxin. Note that the quality of products from these millers is not subject to formal control by

National Regulatory Bodies like the Kenya Bureau of Standards (KEBS).

Observations would indicate that peanuts in Kenya are normally consumed after roasting at the domestic level in a griddle from clay or mettle. Using the same devices, the peanuts are also roasted for selling by street vendors, especially in urban areas like Nairobi. At the industrial level, the peanuts are roasted using commercial roaster to produce a variety of roasted peanut variants which are packaged for sale in the formal markets. At this level also the peanuts are roasted and shelled for manufacture of peanut butter. It has been established that roasting with all types of roasters including microwave oven does not reduce the aflatoxin level very much, explaining the reason why Kenyan peanut butter has always been found to contain levels of aflatoxin higher than the tolerance. Obviously, the roasted unshelled peanut variants would be subsumed to have even higher levels of the toxin than the peanut butter.

The treatments with water and lime were found to lower down the aflatoxin levels in raw peanuts in the present study. The findings of the present study would help in processing peanuts in a commercial scale. The use of UV irradiation

though found effective may find application at the industrial level, but more studies needs to be carried out to establish the safety of the residues of aflatoxin degradation which is currently in dispute [15].

4. CONCLUSIONS

The mean moisture contents of the market peanuts were not significantly different from that recommended for optimum storage to prevent growth of toxin producing moulds, but the mean aflatoxin content was much higher than the tolerance, with about 45% of samples having more than the tolerance. The aflatoxin content of the peanuts was positively and significantly correlated with the moisture content.

Treatment of the peanuts with water (cold & warm) and warm lime at concentrations ranging from 0.01 – 0.04% significantly reduced the aflatoxin to below the tolerance in less than 5 minutes for all treatments. However, the effectiveness of reduction was in the order Calcium hydroxide (lime) > warm water > cold water. Treatment of the peanuts with UV irradiation also managed to reduce the toxin's level to below the tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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