

*Full Length Research Paper*

# **Aflatoxins contamination in raw and roasted cashew nuts in Mtwara, Tanzania**

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The aim of this work was to determine the contamination levels of raw and roasted cashew nuts sold in Masasi and Newala districts of Mtwara region in Tanzania. A total of 60 samples including 40 roasted (24 samples from Newala and 16 from Masasi) and 20 raw samples (12 from Newala and 8 from Masasi) were collected. Determination of total aflatoxins levels in raw and roasted cashew nuts samples was carried out by immune affinity high performance liquid chromatography (HPLC). The levels of contamination ranged from not detected (less than limit of quantification) to 3.29 µg/kg for both aflatoxin B1 and total aflatoxin in the cashew nuts samples. None of the samples had total aflatoxins contamination greater than the recommended maximum residues of 4 µg/kg set by European Commission (2010) or 10 µg/kg set by FAO and WHO (1995). About a quarter (38%) of the samples had total aflatoxins less than limit of quantification. All roasted cashew nut samples were found to have total aflatoxins less than 3 µg/kg while about 86% of raw cashew nut samples had total aflatoxins less than 3 µg/kg.

**Key words:** Aflatoxin, cashew nuts, contamination, Mtwara, Tanzania.

## **INTRODUCTION**

Cashew nuts (*Anacardium occidentale L.*) is derived from South American Countries (Bolivia, Brazil, Ecuador, and Peru) and it is one of the extremely important tropical fruit crops. A cashew fruit consists of an apple that bears fruit in which the kernel is embedded. It is widely cultivated in tropical regions all over the world and it is mainly centralized in third world countries like India, Tanzania, Mozambique, Nigeria, Guinea-Bissau and Kenya (Gong et al., 2016).

Tanzania is among the World's largest producer of raw cashew nuts, whereby in 2017/18, total production of raw

cashew nuts was 313,826 metric tons where by 90% of cashew nuts were exported in raw form due to the country's low processing capacity (TIC, 2019). Cashews are grown mainly in Mtwara, Lindi, Ruvuma and Pwani, which occupies a total plantation area of about 695,683 Ha. About 90% of the area planted with cashew nuts is found in three regions of Mtwara, Lindi and Pwani (Tanzania Investment Centre (TIC), 2019). Most of cashew nuts processing in Tanzania is done manually mainly by small scale processors (CBT, 2018). The domestic consumption of cashew nuts had increased due

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to improved distribution systems through street vendors, shops, mini markets and supermarkets (CBT, 2018). Much of the production is concentrated in a few districts such as Tandahimba, Newala, Masasi, Mtwara, Mkuranga and Nachingwea. It provides an important source of income for 250,000 smallholder farmers in Tanzania, their production accounts for 80-90% of Tanzania's marketed cashew nuts crop. The average smallholder cashew farmer occupies about one to two hectares of cashew nut trees; sometimes intercropped with food crops, mainly cassava, grain staples and legumes (FARMER & CASES, Smallholder Cashew Business Model in Tanzania: Lessons from the Tandahimba Newala Cooperative Union (TANECU) Ltd).

Cashew nuts can be vulnerable to pre and/or post-harvest molds attack due to its high nutritional content but may be accelerated by inappropriate marketing and storage conditions (El-Samawaty et al., 2013). Also environmental factors like humidity and temperature during storage influence the infestation by fungi and aflatoxins production (Hedawoo and Bijwe, 2018). Some type of mold produces highly toxic secondary metabolites known as aflatoxins, which can occur in both industrialized and developing countries when the environmental, social and economic conditions combine with humidity and temperature favor the growth of moulds (Ashraf, 2012). Cashew nuts infection by toxigenic fungi has been reported in a number of studies and revealed a high risk due to contamination with mycotoxins (Alhussaini, 2012; Ashraf, 2012; Adetunji et al., 2018; El-Samawaty, 2013). The mold that attacks cashew nuts are *Aspergillus* species, which produces secondary metabolites known as aflatoxins and it has carcinogenic, estrogenic, immunosuppressive and teratogenic effects in humans and farm animals (Adetunji et al., 2019). There are about eighteen types of aflatoxins that have been identified, but the naturally occurring and well-known forms are AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> (Adetunji et al., 2018).

The Food and Agricultural Organization (FAO) reported 25% of foodstuffs available worldwide are contaminated with mycotoxins, with aflatoxins being identified as the most toxic of these mycotoxins (Eskola et al., 2020). Several countries have been conducting researches on the occurrence of aflatoxins contamination in cashew nuts including Saudi Arabia, Nigeria, South Africa and Brazil with findings revealing levels above but some below the Codex Alimentarius and European Union (EU) limits (El-Samawaty et al., 2013; Adetunji et al., 2018; Adetunji et al., 2019). A study conducted in Nigeria on microbiological quality and risk assessment for Aflatoxins in groundnuts and roasted cashew nuts meant for human consumption showed consumers were at a risk of exposure to foodborne diseases and aflatoxins contamination (Adetunji et al., 2018). Another study showed that cashew nuts were susceptible to fungal deterioration and possibly aflatoxins contamination

especially during storage, AFB<sub>1</sub> was found in 92.3% of cashew nut samples (Ashraf, 2012). A study comparing the fungal metabolite profile of cashew nuts from two African countries (Nigeria and South Africa) showed total aflatoxins of 0.03 to 0.77 µg/kg and 0.01 to 0.28 µg/kg (Adetunji et al., 2019).

In Tanzania different studies have been conducted on the level of aflatoxins such as in maize, cereal based complimentary flour, groundnuts, cereal flours and milk (Nyangi et al., 2016; Rushunju et al., 2013, Mohammed et al., 2016). Although cashew nut is one of the major cash crops in South East and Northern Coastal belt of Tanzania (Annual Agriculture Sample Survey crop and Livestock report, 2016/17) currently, there is limited documented information on the status of aflatoxins contamination. This research aimed at determining the levels of aflatoxins contamination in raw and roasted cashew nuts from Mtwara region, which had the highest production of about 191,025 tonnes, which is 49.2% of all the production in Tanzania (Annual Agriculture Sample Survey crop and Livestock report, 2016/17). The results of this study will provide information on levels of cashew nuts contamination by aflatoxins and contribute to raise awareness and efforts of food control authorities in developing strategies to ensure public safety.

## MATERIALS AND METHODS

### Sample collection

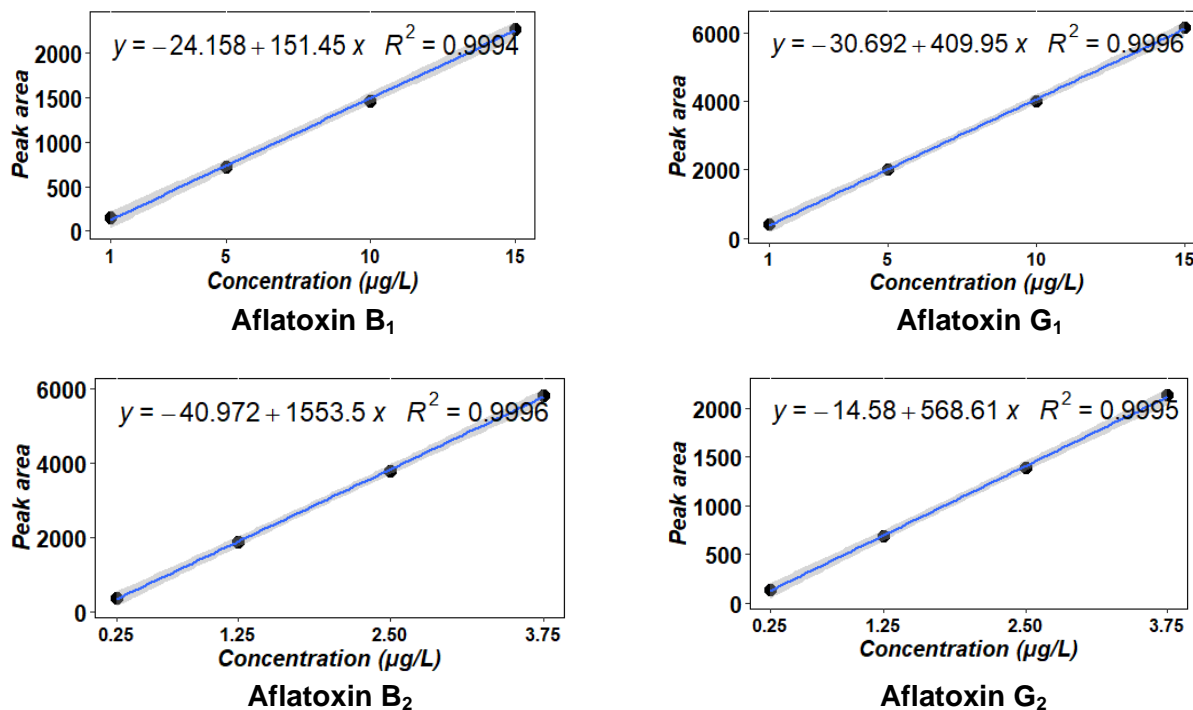
A total of 60 samples of roasted and raw cashew nuts were collected in January 2021, in two districts of Mtwara region; Masasi and Newala. Therefore, 20 raw cashew nut samples and 40 roasted cashew nuts were sampled due to the fact that roasted cashew nuts are mostly consumed. Same as Newala which is divided into 16 wards, sampling was carried out in different wards due to the availability of cashew nuts processors as compared to other wards (Mpita, 2014). The collected samples were packaged in a clean 200 g zipped plastic bags and transported to the Tanzania Bureau of Standards food laboratory in Dar es Salaam for analysis.

### Sample preparation

Each cashew nuts sample (150 g) was ground using a mechanical homogenizer (Hsiangtai grinding machine model SM-450L, serial number 080684) and sub divided to obtain a representative sub-sample for analysis. Then aflatoxins were extracted from 25±0.1 g for each grounded by adding 100 ml of 70:30 methanols:water into the Erlenmeyer flask containing the sample. The flask was covered by aluminium foil then the mixture was shaken by using orbital shaker (SSL1) for 30 min at 250 rpm. The extract was filtered using a filter paper (Whatman 1 circles 125 mm ø). Then 4 ml of the extract was diluted by eight milliliter of distilled water (MillQue, distillate, Elix technology model) into the Teflon tube then vortex for 30 s by using vortex (Tabloys Advanced vortex mixer).

### Immunoaffinity chromatogram

Clean up stage followed whereby the diluted extract was allowed to pass through the immunoaffinity columns (RomerLab, Austria)



**Figure 1.** The calibration curves for aflatoxin standard curves.  
Source: Authors

which are attached to the closed adapter by gravity, then the column was rinsed twice with distilled water the second rinse by using vacuum pressure at the end of the cleanup stage the column was removed from the adapter.

The vials were placed under the column for collection of eluent. A 0.5 ml × 2 ml of ethanol HPLC grade was used to elute the bonded aflatoxins. Then 0.3 ml of eluate was mixed with 0.6 ml of water and 0.1 ml acetonitrile and the mixture was vortexed for 30 s by Talboys advanced vortex mixture set at the speed of 2500 rpm. The sample was injected into the HPLC for quantitative determination of Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

#### Method validation

The HPLC method used was validated by evaluating its linearity, accuracy and sensitivity. The accuracy of the method was determined by spiking of cashew nut sample which was free from aflatoxins contamination and calculating the percentage recovery. About 25 g of aflatoxins free cashew nut samples were spiked with AFB<sub>1</sub> standard at 5 µg/kg. The sensitivity of the methodology or system used was evaluated by limit of detection (LOD) and limit of quantification (LOQ). The limits of detection (LODs) were calculated as concentrations whose peaks were three times the peaks of signal to noise (S/N) ratio, whereas the corresponding limits of quantification (LOQs) were calculated as concentrations using the peaks which were ten times the peaks of signal to noise (S/N) ratios (Saadati et al., 2013).

#### Quality control

The evaluation of the reliability of results, in spite of using validated methods was conducted. The method was found to have a very

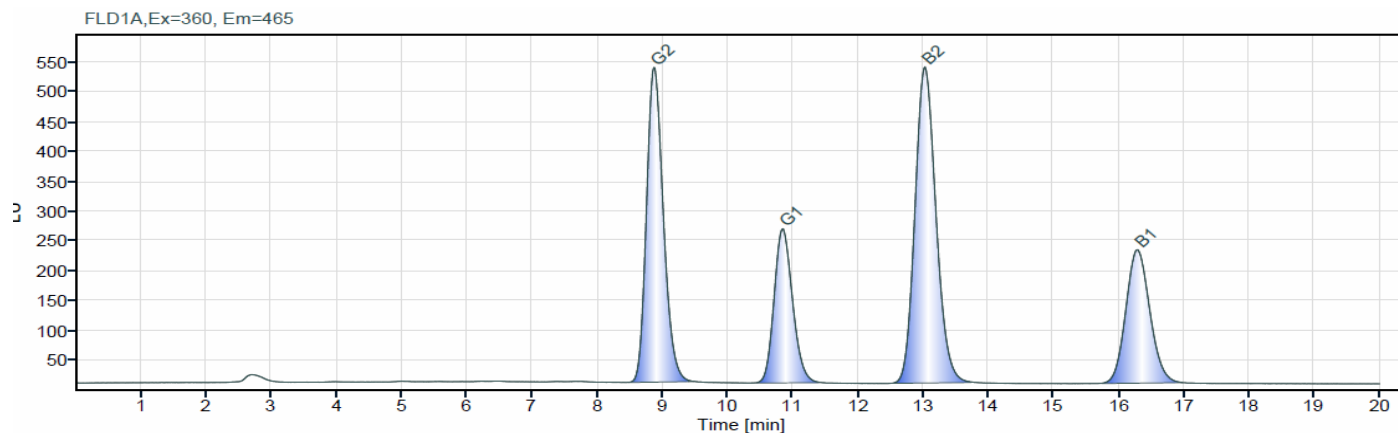
good separation in different aflatoxins as shown in Figure 2. The recovery of aflatoxins ranged from 92.6 to 102.4% which indicated that the method was suitable for aflatoxins analysis. This recovery is within the acceptable recovery range of 90 to 110% (SANTE, 2020).

The limit of detection and quantification for determination of aflatoxins in cashew nuts method by HPLC ranged from 0.13 to 0.16 and 0.16 to 0.29 respectively. All samples that were found to have aflatoxins levels below the detection limit were termed as not detected results.

The linearity of the method was obtained by plotting the instrument response (peak areas) against concentration (µg/L) from four known concentration of aflatoxins standards. The results shows that all calibration curves had strong linear relationship (>0.999) between peak area and concentration as shown in Figure 1. This linear relationship was higher than the minimum acceptable level of 0.998 (Christian, 2007).

#### HPLC analysis of aflatoxins in cashew nuts

A mixture of aflatoxins standard solution B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> of the following concentration 2.02, 2.01, 0.5 and 0.503 µg/ml respectively was used for calibration (Biopure lot number 16192N, Romer Labs, Austria). The diluent was the same as the mobile phase (Water 6: methanol 3: acetonitrile 1). The concentration used was 0.25, 1.25, 2.5 and 3.75 µg/L for B<sub>2</sub> and G<sub>2</sub>; 1, 5, 10 and 15 µg/L for B<sub>1</sub> and G<sub>1</sub>. HPLC coupled with fluorescence detector (serial number: DE60558333, model: G1321A), Pump (serial number: DE62976952, Model: G1311A), Auto sampler (serial number: DE647710, model: G1329A), column oven (serial number: JP94178283, model: G1322A) all from Agilent technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA) were used to analyze the standards and the extracted samples.



**Figure 2.** Chromatogram of different aflatoxins from standard (10 µg/L for B1 and G1; 2.5 µg/L for B2 and G2).  
Source: Authors

**Table 1.** Demographic, general information and awareness of aflatoxins in small scale cashew nuts dealers in two districts of Mtwara region.

Category	Sub-category	Number of observation (%)	Aflatoxin knowledge (%)
Gender	Male	36	9
	Female	64	18
District	Newala	49	10
	Masasi	51	20
Education level	Primary	58	6
	Secondary	39	28
	University/tertiary	3	33
Type of respondent	Processor	1	1
	Consumer	43	0
	Both	56	25
Type of end product	Raw	3	50
	Roasted	6	0
	Both raw and roasted	91	15

Source: Authors

The column C18, ZORBAX Rx-C18 4.6 × 250 mm, 5 µm was used to separate groups of AF B<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> at the column temperature of 300°C and flow rate of 1.2 ml/min. The injection volume of the extracted samples and standard solution was 50 µL. Derivatization of AFG<sub>1</sub> and AF B<sub>1</sub> was conducted after separation with UVA photo ion to allow their detection with fluorescence detector at an emission wavelength of 465 nm and an excitation wavelength of 360 nm.

Readings from the HPLC machine were transformed into peaks and from the peaks to data through computer programmed with LC/MSD Chemstation software Revision B. 04.02 SP1 (212) connected to the HPLC machine.

Quality control was done by running quality control material aflatoxins in corn with the concentration of Aflatoxin B<sub>1</sub>: 8.8 ± 3.1 µg/kg, B<sub>2</sub>: < 1 µg/kg, G<sub>1</sub>: < 1 µg/kg, G<sub>2</sub>: < 1 µg/kg. A blank sample

was prepared using distilled water which was prepared using Evoqua Water Technologies PTE LTD Farrernberg-Germany. The method was found to have a very good separation in different aflatoxins. The recovery of aflatoxins ranged from 92.6 to 102.4% which is within the acceptable recovery range of 90 to 110% (SANTE, 2020). The limit of detection and quantification of aflatoxins in cashew nuts by HPLC ranged from 0.13 to 0.16 and 0.16 to 0.29 respectively. All samples that were found to have aflatoxins levels below the detection limit were termed as not detected results.

#### Statistical analysis

Statistical Package for Social Sciences (IBM SPSS® Version 25

(2017) was used for calculating frequencies and descriptive summaries on data for awareness of aflatoxins contamination. Data on levels of aflatoxins contamination in cashew nuts were analyzed with using R- version 4.0.3 (2020). Analysis of variance (ANOVA) was used to test for significant differences on aflatoxins in raw and roasted cashew nuts from different districts. Mean separation test was done by Turkey HSD multiple rank test with agricolae package.

## RESULTS AND DISCUSSION

The demographic characteristics of cashew nuts processors and cashew nuts consumers on awareness, handling practices and factors associated with aflatoxins contamination in cashew nuts were investigated and the levels of contamination were evaluated.

### Awareness on aflatoxins and factors associated with aflatoxins contamination in cashew nuts

Demographic characteristics of cashew nuts dealers showed that most of them were female (64%) and education level ranging from primary school (58%), secondary school (39%) and few had tertiary/university education (2.5%). A study in India revealed that the cashew industry provides employment to a large number of poor women workers from rural areas (Pattanayak, 2020). Most of them were both consumers and processors of both raw and roasted cashew nuts as shown in Table 1.

Generally, very few respondents (<20%) in either category had heard aflatoxins in their lifetime. Almost 30% of all respondents who heard the word aflatoxins, heard it during different trainings. For example, more than 50% of all respondent had primary school education, only 6% had heard the word aflatoxins whereas 28% of respondents who had secondary school education had heard about aflatoxins. Similar findings in a study on the awareness of mycotoxins infections in Kilosa district of Tanzania found out that respondents with low level of education (below secondary level) were 1.805 times more likely to have low level of awareness and knowledge than those who had higher education (Magembe et al., 2016).

In addition to these respondents who were aware of aflatoxins, all were also aware that aflatoxins is caused by fungi, cashew nuts can be contaminated by aflatoxins and poor storage might be a cause of fungal growth and thus contaminated with aflatoxins and eating contaminated cashew nuts can cause illness or death. One cashew nuts respondent who was aware of aflatoxins responded that poor air circulation in storage conditions can results to fungal growth while the remaining 16 dealers mentioned that high moisture content during storage of cashew nuts can results to fungal growth. More than 60% of the processors produce both roasted and raw cashew nuts (Table 1) but roasted cashew nuts are highly consumed than raw (more than

50%) (Table 2). Most of the cashew nuts processors are also good consumers of the cashew nuts (>50%).

### Storage practices of cashew nuts

The cashews are stored in the form of shelled cashew nuts, processing is done batch wise and especially when one receives an order for processed cashew nuts. Processed cashew nuts are not kept for more than six month before they are sold; more than 40% processors from Newala and more than 50% processors from Masasi were storing the processed cashew for not more than three month (Table 3).

Almost all the processors owned a farm where their raw materials come from, only few (<20%) obtain their raw materials from other farms. A study done by Azam-Ali and Judge (2001) showed an estimation of 280,000 households, covering an area of 400,000 ha, is involved in cashew production and the government is actively supporting them in improving the condition of the trees and maximizes agronomic potential. The processed cashew nuts are kept in either plastic buckets or in plastic bags ready to be sold. All the respondents were storing the shelled cashew nuts in jute bags.

Most of the products from street vendors were found packed in transparent nylon bags with or without labels and some of them were found not packed at all. On the other hand, the local processors were found keeping the processed cashew nuts in plastic buckets prior to packing in zipped plastic bags of different sizes ready for selling; details were on the label of some of the packages whereas other packages lack the details. A study done by Ramadhani et al. (2014) found similar scenario where plastic buckets (87.5%) and other materials such as paper boxes (12.5%) but for street, vendors' plastic films (polyethylene bags) were the main packaging materials used (97.5%).

A study done by Ramadhani et al. (2014) on the physicochemical quality of street vended roasted cashew nuts in Tanzania also found that immediately after roasting, cashew nuts were stored in plastic buckets (87.5%) and other materials such as paper boxes (12.5%). A study to evaluate the effect of packaging materials on moisture and microbiological quality of roasted cashew nuts revealed that plastic and glass bottles had counts within the acceptable limits (Oladapo et al., 2014).

About a quarter (38%) of the samples had total aflatoxins less than limit of quantification (0.16 µg/kg) while all roasted cashew nuts from both districts had total aflatoxin less than 3 µg/kg (Table 4). All roasted cashew nut samples were found to have total aflatoxins less than 3 µg/kg while about 86% of raw cashew nut samples had total aflatoxins less than 3 µg/kg. None of the samples had aflatoxins contamination greater than recommended maximum residues of 4 µg/kg set by European Commission (2010) or 10 µg/kg set by FAO and WHO

**Table 2.** Eating practices for cashew nuts dealers in Mtwara region.

Category	Response	Newala (%)	Masasi (%)
Frequency	Rarely	12	7
	Sometimes	29	30
	Daily	59	63
Amount eaten (g)	100	25	38
	200	68	43
	More than 300	7	19
Type eaten	Raw	0	2
	Roasted	54	62
	Backed	12	10
	Any type	34	26

Source: Authors

**Table 3.** Storage information for cashew nuts dealers in Mtwara region.

Category	Sub-category	District	
		Newala (%)	Masasi (%)
Storage time (months)	1-3	49	73
	3-6	49	16
	More than 6	2	11
Source of raw materials	Other farms	23	13
	Own farm	60	68
	Own farm and others	17	19
Storage area	Bare ground	0	3
	Jute bag	100	97
Storage type	Plastic bags	47	47
	Plastic buckets	53	26

Source: Authors

(1995) for similar products such as pistachio and almond. In both districts, raw cashew nut samples had high levels of total aflatoxins than roasted cashew nut samples indicating that roasting reduces the levels of contamination.

#### Aflatoxins contamination within the districts

The levels of aflatoxins in cashew nuts (raw and processed) are expressed in Table 5. Statistical difference was observed in aflatoxin B<sub>1</sub> and total aflatoxins. Raw cashew nuts were found to have statistically higher values of total aflatoxins compared to roasted cashew nuts in both districts. For those samples where aflatoxins were detectable, aflatoxin B<sub>1</sub> was generally the major

contributor to total aflatoxins.

The levels of aflatoxins in this study was found to be higher than the levels found in the studies done in Nigeria and South Africa (Adetunji et al., 2019) that found total aflatoxins in cashew nuts to be between 0.28 and 0.77 µg/kg respectively. High levels of aflatoxins in cashew nuts (31.50 µg/kg) were detected in a study done in north eastern Brazil in 2010 (Milhome et al., 2014).

Another study done in Vietnam on multi-mycotoxin (18 toxins) in cashew nuts showed a high level of contamination of up to 32.1 µg/kg for aflatoxin B<sub>1</sub> (Le et al., 2021). Effect of roasting on degradation of aflatoxins have been observed in different crops such as pistachio (Yazdanpanah et al., 2005), soybeans (Hamada and Megalla, 1982), peanut (Martins et al., 2017) and other crops that have been reviewed by Emadi et al. (2021).

**Table 4.** Aflatoxin ( $\mu\text{g}/\text{kg}$ ) contamination in Masasi and Newala districts in raw and roasted cashew nuts.

District	Process	Range B <sub>1</sub>	Range TAF	TAF <LOQ	TAF <3 %	3<TAF<5
Masasi	Raw	ND - 3.29	ND - 3.29	25	88	12
	Roasted	ND - 2.17	ND - 2.36	63	100	0
Newala	Raw	0.96 - 3.18	1.17 - 3.24	0	83	17
	Roasted	ND - 2.59	ND - 2.78	46	100	0

Source: Authors

**Table 5.** Aflatoxin contamination ( $\mu\text{g}/\text{kg}$ ) in raw and roasted cashew nuts from Masasi and Newala districts.

District	Process	Aflatoxin G <sub>2</sub>	Aflatoxin G <sub>1</sub>	Aflatoxin B <sub>2</sub>	Aflatoxin B <sub>1</sub>	Total
Masasi	Raw	0.05±0.02 <sup>a</sup>	0.01±0.02 <sup>a</sup>	0.02±0.01 <sup>a</sup>	1.71±0.43 <sup>a</sup>	1.79±0.43 <sup>a</sup>
	Roasted	0.04±0.01 <sup>a</sup>	0.01±0.02 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.83±0.12 <sup>b</sup>	0.90±0.12 <sup>b</sup>
Newala	Raw	0.04±0.02 <sup>a</sup>	0.03±0.02 <sup>a</sup>	0.03±0.01 <sup>a</sup>	2.02±0.23 <sup>a</sup>	2.12±0.22 <sup>a</sup>
	Roasted	0.04±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.87±0.13 <sup>b</sup>	0.97±0.14 <sup>b</sup>

Means with different letters within the column are statistically significant ( $p < 0.05$ ).

Source: Authors

The lower value of aflatoxins in roasted cashew nuts can be accounted for by application of heat during heating that can degrade the toxins or enhance the reactions between the aflatoxins and other compounds of the cashew nuts and might modify the structures of the toxins (Farahmandfar and Tirgarian, 2020).

The higher value of aflatoxins in raw cashew nuts is linked principally to water activity ( $a_w$ ). This observation is attributable to improper drying which predisposes stored produce to growth of mycotoxigenic fungi such as *Aspergillus* species which is conjectured to also increase with storage time (Temba et al., 2017).

## Conclusion

Determination of the levels of aflatoxins contamination in the two districts of Mtwara region in Tanzania showed that in all samples, AFB<sub>1</sub> and total aflatoxins levels were below the maximum limit recommended by the European Commission regulations (4  $\mu\text{g}/\text{kg}$ ) as well as for the Tanzania Bureau of Standards (10  $\mu\text{g}/\text{kg}$ ). The study revealed that raw cashew nuts were more contaminated than roasted cashew nuts in both districts suggesting that roasting is one way of reducing contamination. Aflatoxicosis is still one of the main public health concerns in Tanzania that lead to health hazards in the population. There is a need to reduce the contamination by controlling aflatoxins contamination through Good Agriculture Practices (GAP) at farm level as well as improved storage conditions. It is important that farmers,

processors and everyone who are involved in cashew nuts value chain to be educated on the potential carcinogenic nature of the aflatoxins in human health.

## Recommendations

The small scale cashew nuts processors do not have any instrument for moisture content determination and the removal of the outer and inner coat of the nuts is being done by bare hands, therefore the authorities should take the lead in the efforts to establish mandatory regulations in cashew nuts farming, processing and storage to decrease contamination risks to toxigenic fungi. Also strict hygienic measures should be implemented during storage, drying and packing so as to minimize contamination, this will enhance international trade efforts and improved public health.

## CONFLICT OF INTERESTS

The authors disclose no conflicts of interest.

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