AFLATOXINS CONTAMINATION IN RAW AND LOCALLY PROCESSED CASHEW NUTS AND THE ASSOCIATED HUMAN HEALTH RISKS: A CASE STUDY OF MASASI AND NEWALA DISTRICTS

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EXTENDED ABSTRACT

Aflatoxins (AFs) are one of the mycotoxins which are secondary metabolites of various fungi species; *Aspergillus flavus, A. parasiticus* and *A. nomius*. There are about eighteen types of aflatoxins that have been identified, but only four are found in food and feed: B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most toxic and classified as group one carcinogen. Aflatoxins cause serious health problems and have high potential effects on liver cancer, therefore, numerous investigations have been conducted worldwide. 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, evaluate the factors associated with the contamination and estimate human exposure to aflatoxin through consumption of contaminated cashew nuts. Determination of total aflatoxins levels in raw and roasted cashew nuts samples was carried out at Tanzania Bureau of Standards (TBS) food laboratory in Dar es Salaam by using immuno-affinity high performance liquid chromatography. A total of 60 samples were collected and the highest concentration of aflatoxins recorded for raw cashew nuts was 3.29 µg/ kg in Masasi and 3.24 µg/kg in Newala. 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. All roasted cashew nut samples had total aflatoxins less than 3 µg/kg while about 86% of raw cashew nut samples had total aflatoxins less than 3 µg/kg. The overall results of the tested samples indicated that the rate of contamination was very minimal for cashew nuts especially roasted nuts but raw cashew nuts showed higher contamination. A cross-sectional survey was conducted to assess awareness among cashew nuts stakeholders (smallholder farmers, traders and consumers) on aflatoxins contamination, demographic characteristics, handling practices and eating habit. The study showed that the cashew nuts value chain is highly dominated by females (64%), whose education level was primary school (58%). Very few respondents (<20%) in either category have heard of aflatoxins in their lifetime or almost all were among those who have had either a secondary school or a college level of education. This study revealed that education level of the respondents was directly related to aflatoxins awareness.

The human exposure to aflatoxin through consumption of contaminated cashew nuts in the study area was also estimated, whereby data on the rate of consumption was obtained through a structured questionnaire which involved 120 respondents. The results were used to calculate the Estimated Daily Intake (EDI) and the Hazard Index (HI). The risk assessment for aflatoxins was evaluated by the qualitative Margin of Exposure (MOE) approach and the quantitative hepatocellular carcinoma (HCC) risk approach. The estimated cancer risk of hepatocellular carcinoma (HCC) in this study ranged from 0.006 to 0.394 cancers per year per 10^5 individuals, upon consumption of cashew nuts with aflatoxin B_1 greater or equal to LOQ to highest contaminated samples, respectively. Since roasted cashew nuts which have less contamination level are highly consumed than raw (more than 50%), exposure to aflatoxins is very minimal to cashew nuts consumers. Although estimation of the margin of exposure and risk HCC revealed that cashew nuts, may pose health risks to consumers depending on the rate of consumption of the contaminated cashew nuts and the amount consumed.

DECLARATION

I, Catherine Leonard Akwilapo do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFs	Aflatoxins
ANOVA	Analysis of Variance
BMDL	Benchmark dose lower limit
CBT	Cashew nut Board of Tanzania
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agricultural Organization
FQ	First Quartile
GAP	Good Agricultural Practices
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HPLC	High performance Liquid Chromatograph
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
Kg	Kilogram
L	Litre

LC/MSD	Liquid Chromatography/ Mass Selective Detection
LCR	Liver Cancer Risk
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Mean
mg	Milligram
ml	Millilitres
MOE	Margins of Exposure
Ng	Nanogram
PMTDI	Provisional Maximum Tolerable Daily Intake
SC	Scientific Committee
TAF	Total Aflatoxin
TANECU	Tandahimba Newala Cooperative Union
TBS	Tanzania Bureau of Standards
TIC	Tanzania Investment Centre
TQ	Third Quartile
UNIDO	United Nations Industrial Development Organization
USAID	United States Agency for International Development
WHO	World Health Organization
μg/kg	Microgram per kilogram (ppb)

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Cashew nut (*Anacardium occidentale L.*) has originated from Brazil and it is very important tropical fruit crops (Gong *et al.,* 2016). Cashew fruit consists of an apple that bears a fruit in which the kernel is embedded. It is cultivated in tropical regions all over the world, including countries like, Tanzania, India, Nigeria, Mozambique, Guinea-Bissau and Kenya (Gong *et al.,* 2016). The cashew kernel contains approximately 40-57% fat as a major nutritional composition followed by 26% carbohydrate, 21% protein and 1.5% mineral matters (Rico *et al.,* 2016).

Tanzania is one of the highest cashew producers in Africa, and the main production areas are the south-eastern part of Tanzania including Lindi and Mtwara region, the coastal region and Ruvuma region in the southern part of Tanzania (Cashew Board of Tanzania, 2018). Although cashew processing in Tanzania provides jobs to low-skilled labors, especially women but only 40% of the cashew nuts produced in Tanzania are processed in the country, the remaining is for export market (Tanzania Investment Centre, 2019).

Cashew nut can be vulnerable to pre- and/or post-harvest moulds attack due to its high nutrient content and this can also be accelerated by inappropriate marketing and storage conditions (El-Samawaty *et al.,* 2013). It has also been shown, elsewhere, that environmental factors like humidity and temperature during storage greatly influence the contamination by fungi and aflatoxins production (Hedawoo and Bijwe, 2018). Cashew nuts infection by toxigenic moulds has been researched in a several studies worldwide and revealed a high risk due to contamination with mycotoxins (Alhussaini, 2012, Ashraf, 2012, Adetunji, 2018, El-Samawaty, 2013). The moulds that attack cashew nuts are *Aspergillus* spp which produce secondary metabolites known as aflatoxins which have estrogenic, immunosuppressive, carcinogenic, 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 aflatoxin B_1, B_2, G_1 and G_2 (Adetunji *et al.,* 2018).

1.2 Cashew nuts production and consumption in Tanzania

Among the cash crops in Tanzania cashew nuts is one of very important in the export market and it ranks the fourth in the country which is important to the country's economic growth and also one of the vital source of income to small scale farmers because it provides employment for the workers in the farms and processing chain (CBT, 2013).

In 2017/2018 the total production of raw cashew nuts in Tanzania was 313,826 metric tons whereby 90% of cashew nuts were exported in raw form due to the country's low processing capacity, this makes Tanzania among the largest producers of raw cashew nuts in the world (Tanzania Investment Center, 2019). The plantation area of cashew nut is about 695,683 hectares and is mainly in the Southern Tanzania region (Lindi, Mtwara, Ruvuma and Coastal region). About 90 % of the area planted with cashew nuts is found in Mtwara, Lindi and Coast region (Tanzania Investment Centre, 2019).

In Tanzania cashew nut processing is concentrated in a few districts including Newala, Tandahimba, Masasi, Mkuranga and Nachingwea and it is carried out manually mainly by small- scale processors (CBT, 2014). Almost half a million Tanzanians are engaged in small-scale cashew nuts farming, particularly in the south- eastern part of the country, including Lindi and Mtwara region (Farmer and Cases, 2012). An average smallholder cashew farmer grow about 1-2 hectares of cashew nut trees which are sometimes intercropped with food crops, mainly cassava, grain staples and legumes so the crop provides an important source of income for 250,000 smallholder farmers in Tanzania and their production accounts for 80-90% of Tanzania's marketed cashew nut crops (Farmer and Cases, 2012). Although cashew nut is highly cultivated in Tanzania the domestic consumption has been very low but it has increased due to a better distribution systems where street vendors, mini markets, shops, and supermarkets are also selling cashew nuts (CBT, 2013).

1.3 Occurrence of aflatoxins in cashew nuts

Globally nuts are one of the most common snacks but their poor handling has made them prone to aflatoxins contamination and this poses a serious challenge on the health of the consumers (CBT, 2014).

The occurrence of mycotoxins in foods is associated with climatic conditions of a given area, agronomical practices and crops grown (Wu *et al.*, 2016). Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing can also contribute to fungal growth (Hedawoo and Bijwe, 2018). The mycoflora contaminations which are the most frequent species recovered from cashew nuts are *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Mucor* spp. and *Syncephalastrum* sp. (El-Samawaty *et al.*, 2013). *Aspergillus, Penicillium* and *Fusarium* are considered the most serious fungal genera which contaminate cashew nuts due to toxic metabolites. A study which was carried out in Riyadh, Kingdom of Saudi Arabia by using HPLC analysis on Mycotoxins production ability of *A. flavus* and *A. niger* showed that most of *Aspergillus flavus* isolates were capable of producing toxins, meanwhile, 40% were aflatrem (2ppb) producers (El-Samawaty *et al.*, 2013).

1.4 Health impact of aflatoxins on humans

In the 83rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which was held in 2016, the cancer risks which was due to the estimated aflatoxins exposure in different regions was calculated. It showed that sub-Saharan African countries and Haiti had the highest estimate of cancer risk which ranged from 0.21 to 3.94 aflatoxins induced cancers per year and per 100,000 subjects. East Africans are at a high risk of exposures and effects of mycotoxins, possibly it is the area where mycotoxicosis have impacted mankind the most (Kimanya, 2015). The health effects of aflatoxins have been reviewed by a number of expert groups. The International Agency for Research on Cancer (IARC) has concluded that naturally occurring aflatoxins are group 1 carcinogen to humans with a role in aetiology of liver cancer especially on people who are carriers of hepatitis B virus (HBV) surface antigens.

Another outcome which is associated with aflatoxins exposure is the hepatocellular carcinoma (HCC) (Kimanya, 2015), this usually affects children due to its acute hepatotoxicity which results from eating aflatoxins contaminated food and other toxicants. Children are mostly affected to aflatoxins due to their body weight which is lower than the adult body, the metabolic rate which is higher and the incomplete development of inner tissues and organs (Magoha *et al.,* 2016). Apart from HCC the toxin also results to stunting growth and in Tanzania it was reported in Dodoma district whereby children under the age of five were the study group and over 40% stunting was observed which marks the highest stunting levels in the country (TFNC, 2014).

1.5 Factors that influence aflatoxins contamination in cashew nuts

The aflatoxins contamination in food products depends on the range of factors throughout the food chain from farming, harvesting and storage condition. For the case of nuts proper storage condition should be employed during storage such as ventilated area, appropriate temperature, humid conditions and moisture content (Maturová, 2019). The most important factors influencing the growth of fungi during storage of the nuts are the storage temperature, **moisture content** and presence of oxygen (Ramadhani *et al*., 2014). Cashew nuts should be dried to the moisture content of 8-10% because storage with moisture content above 10% will result into decay or rotting of the nuts as well as mould growth (El-Samawaty *et al.*, 2013).

The effect of storage temperature was demonstrated by Amnah and Alsuhaibani (2018) who conducted a study in Saudi Arabia on the prevalence and level of contamination due to aflatoxins B_1, B_2, G_1 and G_2, the mould and yeast counts in nuts specifically walnuts, pistachios, cashews, almonds and hazelnuts and the effect of different storage conditions (storage temperature and storage time) on aflatoxins formation and mould and yeast counts. It was concluded that the amount of aflatoxins was above safe limits in all of the nuts stored for 3 or 6 months at room temperature (25°C) and 45°C.

1.6 Problem statement and Justification

According to FAO, approximately 25% of foodstuffs worldwide are contaminated with mycotoxins, with aflatoxins being identified as the most toxic of these mycotoxins (Eskola *et al.*, 2020).). Due to the toxicity of the aflatoxin different countries have been conducting researches on the occurrence of aflatoxins in cashew nuts including Saudi Arabia (Elzupir *et al.*, 2018), Nigeria (Adetunji *et al.*, 2018; Adetunji *et al.*, 2019), South Africa (Omotayo *et al.*,2019) and Brazil (Yunes *et al.*, 2020). Some of the studies have reported aflatoxin levels above, but some below the Codex Alimentarius and European Union (EU) limits. 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 in Saudi Arabia showed that cashew nuts were susceptible to fungal deterioration and possibly aflatoxins contamination especially during storage, AFB_1 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 0.03-0.77 µg/kg and 0.01-0.28 µg/kg respectively which were below the maximum recommended level (Adetunji *et al*., 2019).

In Tanzania different studies have been conducted on the level of aflatoxins such as in maize (Nyangi *et al.,* 2016), cereal based complimentary flour (Kuhumba *et al.,* 2018), groundnuts (Rushunju *et al.,* 2013), cereal flours, milk (Mohammed *et al.,* 2016). Also a risk assessment study of aflatoxins have been reported by Shirima *et al*., (2015) in three regions (Iringa, Tabora and Kilimanjaro), high prevalence of chronic aflatoxins exposures were found in young children being associated with maize diet, this is because in Tanzania, maize is a staple food which is highly consumed, at an estimate of 400 grams a day per person (Kamala and Kimanya, 2017). Currently, there is limited documented information on the status of aflatoxins contamination of cashew nuts and the risk due to consumption of contaminated cashew nuts. The aim of this study was to determine the level 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 percent of all the production in Tanzania) (TIC and USAID, 2016/17). The study was also intended to make an assessement on the awareness, knowledge and practices associated with aflatoxins contamination in cashew nuts among small scale processors throughout the cashew nuts process chain as well as to estimate the human risk due to consumption of contaminated cashew nuts. The study therefore, serves as the current status of aflatoxins contamination in cashew nuts which help to estimate safety level of consumers in the study area and also provide information and guide policymakers to ensure that the maximum allowable limits established are respected, thereby preventing economic and public health problems.

1.7 General objective

1.7.1 Overall objective

The overall objective of this study was to determine the aflatoxins contamination in raw and roasted cashew nuts in Mtwara region and assessment of associated human health risks.

1.7.2 Specific objectives

The specific objectives of the study were to:

i. Determine the level of aflatoxins in raw and roasted cashew nuts in Masasi and Newala.

ii. Evaluate the factors associated with aflatoxins contamination in cashew nuts.

iii. Estimate of human exposure to aflatoxin through consumption of contaminated cashew nuts.

1.8 List of draft manuscripts

The findings of this research were reported in two manuscripts presented as chapter two and three.

i. Aflatoxins contamination in raw and roasted cashew nuts and the factors associated with the contamination: a case study of Masasi and Newala districts

ii. Estimate of human exposure to aflatoxin through consumption of contaminated cashew nuts: a case study of Newala and Masasi districts.

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CHAPTER TWO

2.0 AFLATOXINS CONTAMINATION IN RAW AND ROASTED CASHEW NUTS AND AWARENESS OF THE FACTORS ASSOCIATED WITH THE CONTAMINATION: A CASE STUDY OF NEWALA AND MASASI DISTRICT

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2.1 Abstract

Cashew nuts ranks 4th among cash crops and export crops in Tanzania, there in a clear need to establish its aflatoxins status as well as awareness status on aflatoxins among the cashew nuts processing stakeholders. The aim of this study was to determine the contamination levels of raw and roasted cashew nuts consumed in Masasi and Newala districts of Mtwara region in Tanzania and assess the factors associated with the contamination. 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. Immunal affinity HPLC was used to determine the level of aflatoxins and a cross-sectional survey was conducted to assess awareness among cashew nut stakeholders (smallholder farmers, traders and consumers) on aflatoxins contamination and handling practices. The levels of contamination ranged from not detected (less than Limit of Quantification) to 3.29 µg/kg for total aflatoxins in cashew nut 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) and the Tanzania Standards (TZS 739: 2010). All roasted cashew nut samples had total aflatoxins less than 3 µg/kg while about 86% of raw cashew nut samples had total aflatoxins less than 3 µg/kg. Raw cashew nuts were higher contaminated as compared to roasted cashew nuts. Very few respondents knew about aflatoxins, the few who knew were the ones who had education of above primary school level. Females were highly involved in the cashew nuts processing and selling. Cashew nuts have very minimal contamination to aflatoxins although even low count of aflatoxins should be handled with care because aflatoxins are genotoxic carcinogens. Policy makers should make sure proper education on the severity of aflatoxins is known by the stakeholder and continual improvement on storage practices in order to guarantee that human exposure levels are kept as low as possible.

Key words: Aflatoxins, cashew nuts, contamination

2.2 Introduction

The incidences of food contamination have become frequent in recent years which raise questions about human health and economic consequences (Ashraf, 2012). Nuts including cashew nuts are considered as food with a number of health benefits, they are prone to contamination by toxigenic fungi which releases metabolites known as aflatoxins (Milhome *et al.*, 2014). Aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungi *Aspergillus flavus*, which produce only B aflatoxin and *Aspergillus parasiticus* which produces both aflatoxins B and G. These toxins were brought to light in the early 1960's as the reason of the turkey X disease epidemic, which resulted in the deaths of thousands of poultry that were fed a toxic contaminated peanut meal (Wu *et al.*, 2014). The aflatoxins B_1, B_2, G_1 and G_2 are the major groups and they are usually found together in various foods and feeds in different proportions (Ashraf, 2012). Therefore, data on the occurrence of aflatoxins in foods and feeds are needed to enable exposure assessment and estimate the effects of regulatory limit (Marin *et al.*, 2013).

Aflatoxins produced by the *Aspergillus* species are highly toxic, carcinogenic and cause severe contaminations to food sources, leading to serious health consequences (Adetunji *et al.*, 2018). Contaminations by aflatoxins have been reported in food and feed, such as groundnuts (Bediako *et al.*, 2019), millet (Keta *et al.*, 2019), sesame seeds (Echodu *et al.*, 2019), maize (Bediako *et al.*, 2019), rice (Prietto *et al.*, 2015), spices (Hammami *et al.*, 2015) and cocoa (Pires *et al.*, 2019) due to fungal contamination in pre- and post-harvest conditions. Besides these food products, commercial products like peanut butter (Maarufu and Mariamu, 2019) and cooking oil (Mmongoyo *et al.*, 2017) have also been reported to be contaminated by aflatoxins. Even a low concentration of aflatoxins is hazardous for human and livestock. The identification and quantification of aflatoxins in food and feed is a major challenge to guarantee food safety. Although cashew nuts is one of the major cash crops in south east and northern coastal belt of Tanzania (TIC and USAID, 2019). Currently, there is limited documented information on the status of aflatoxins contamination of cashew nuts. The aim of this study was to determine the level 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 percent of all the production in Tanzania) (TIC and USAID, 2019).

The present study therefore, determined the level of aflatoxins contamination in raw and roasted cashew nuts commercially available in Newala and Masasi districts of Mtwara region, in Tanzania. This will help to understand safety of the cashew nuts with respect aflatoxin contamination in order to facilitate implementation of appropriate measures for the protection of consumer health and facilitation of cashew nut trade.

2.3 Materials and Methods

2.3.1 Study area

The study was conducted in Masasi and Newala districts in Mtwara region (Tanzania Investment Centre, 2019). The districts were picked because they are among the districts of major producers of the cashew nuts in Mtwara region. In addition the cashew nuts are the major cash crops in the districts (CBT, 2014). The study population was small scale processors of cashew nuts, workers in the cashew processing chain as well as cashew nuts sellers.

2.3.2 Sampling

The study employed probability sampling technique (Adwok, 2015). Processors were randomly selected in their respective wards through simple random sampling technique in which selection of processors based on their involvement in cashew nuts processing and in cashew nuts chain. Other information on the rate of consumption was collected from street vendors and people involved in the processing chain of cashew nuts.

2.3.3 Sample size

The sample size was estimated using Kothari equation (Kothari and Garg, 2014): -

$$n = z^2 P$$

$$\frac{(1-P)}{e^2}$$

Where; n= sample size, Z= standard variate at a given confidence level, for this study a 95% confidence level = 1.96 at and e = acceptable error (the precision/ estimation error) set at 0.12% for this study.

Thus,

$$\frac{1.96\,2 * 0.5(1-0.5)}{0.12^2} = 60$$

A total of 60 samples of which 40 roasted (24 samples from Newala and 16 from Masasi) and 20 raw samples (12 from Newala and 8 from Masasi) were collected. Sampling was carried out in different wards due to the availability of cashew nuts processors as compared to other wards (Mpita, 2014). The samples were bought at the small scale processors and packaged in a clean 200g zipped plastic bags which were coded to differentiate between raw and roasted cashew nuts. Samples were then transported to Tanzania Bureau of Standards food chemistry laboratory in Dar es Salaam for aflatoxins analysis.

2.3.4 Questionnaire Survey

A structured questionnaire was used to collect information of awareness, knowledge and practices associated with aflatoxins contamination in cashew nuts from February to March, 2021 in Masasi and Newala. Information on storage practices of raw and roasted cashew nuts were gathered and the packaging materials were carefully assessed. 120 respondents were randomly selected among the cashew nuts processors and vendors from each districts.

2.3.5 Determination of aflatoxins

Total aflatoxins levels in raw and roasted cashew nut samples were analysed by using High Performance Liquid Chromatography (HPLC) as described ISO 16050:2003.

2.3.5.1 Sample preparation

Cashew nut samples (150 g) were ground using a mechanical homogenizer (Hsiangtai Grinding Machine model SM-450L, serial number 080684, China) and sub divided to obtain a representative sub-sample for analysis. Then aflatoxins were extracted from 25 ± 0.1g for each grounded by adding 100 ml of 70: 30 methanol: 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 minutes at 250 rpm. The extract was filtered using filter paper (Whatman 1 circles 125 mm ø). Then four ml of the extract were diluted by eight milliliter of distilled water obtained from MillQue, distillate, Elix technology model (USA) into the Teflon tube then vortex for 30 seconds by using vortex (Tabloys Advanced vortex mixer).

2.3.5.2 Immunoaffinity chromatogram

Clean up involved passing by gravity the diluted extract through the immunoaffinity columns (RomerLab, Austria) which are attached to the closed adapter. Then the column was rinsed twice with distilled water and the second rinse by using vacuum pressure until the end of the cleanup stage when the column was removed from the adapter. Eluents were collected in vials placed under the column.

Ethanol HPLC grade 0.5 ml x 2 ml of 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 second at the speed of 2500 rpm. The sample was injected into the HPLC for quantitative determination of aflatoxin B_1, B_2, G_1 and G_2.

2.3.5.3 HPLC analysis of aflatoxins in cashew nuts

A mixture of aflatoxins standard solution B_1, B_2, G_1, G_2 (Biopure lot number 16192N, Romer Labs, Austria) of the following concentration 2.02 µg/ml, 2.01 µg/ml, 0.5 µg/ml, 0.503 µg/ml respectively was used for calibration. 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 AFB_2 and AFG_2; 1, 5, 10 and 15 µg/L for AFB_1 and AFG_1. HPLC (Agilent technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA) 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. The column C18, ZORBAX Rx- C18 4.6 x 250 mm, 5 µm was used to separate groups of AFB_1, AFB_2, AFG_1 and AFG_2 at the column temperature of 300 ºC and flow rate of 1.2 ml/min. The injection volume of the extracted samples and standard solution was 50 µL. Derivatization of AFG_1 and AFB_1 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. Values 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.

2.3.6 Method validation

The HPLC method used was validated by evaluating its linearity, accuracy and sensitivity. The accuracy of the method was determined by spiking cashew nuts which was free from aflatoxins contamination and calculating the percentage recovery (Table 2.1). About 25 g of aflatoxins free cashew nut samples were spiked with AFB_1 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) (Table 2.2). 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).

2.3.6.1 Quality control

The evaluation of the reliability of results, inspite of using validated methods was conducted. The method was found to have a very good separation in different aflatoxins as shown in Figure 2. 2. The recovery of aflatoxins was ranged from (92.6 to 102.4) % which indicated that the method was suitable for aflatoxins analysis (Table 2.1). This recovery is within the acceptable recovery range of 90 to 110% (SANTE, 2020).

Table 2. 1: Recovery of aflatoxins from spiked cashew nut sample

	Concentration of aflatoxins in blank sample (µg/kg)	Spiked concentration (µg/kg)	Detected concentration (µg/kg)	% Recovery
AFG_2	0.00	5.00	5.10	102.00
AFG_1	0.00	5.00	4.91	98.20
AFB_2	0.00	5.00	5.12	102.40
AFB_1	0.00	5.00	4.63	92.60

The limit of detection and quantification for quantification of aflatoxins in cashewnuts method by HPLC were ranging from 0.13 to 0.16 and 0.16 to 0.29 respectively as shown in Table 2.2. All samples that were found to have aflatoxins levels below the detection limit were termed as not detected results.

Table 2. 2: The Limit of detection (LOD) and limit of quantitation (LOQ) for each analyzed aflatoxins

Compound	LOD (µg/kg)	LOQ (µg/kg)
AFG_2	0.13	0.16
AFG_1	0.13	0.21
AFB_2	0.13	0.18
AFB_1	0.16	0.29

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 as shown in Figure 2.1. The results shows that all calibration curve were having strong linear relationship (>0.999) between peak area and concentration as shown in Figure 2. 1. This linear relationship was higher than the minimum acceptable level of 0.998 (Christian, 2007).

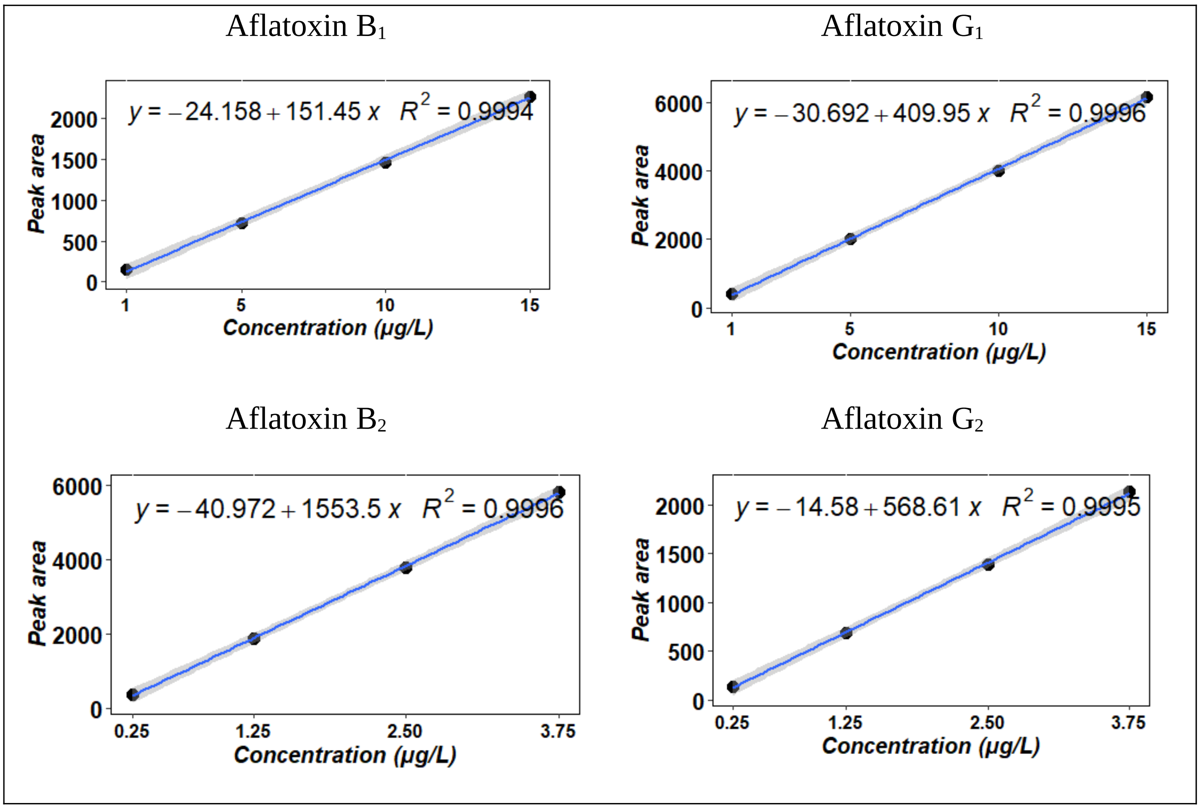

Figure 2. 1: The calibration curves for Aflatoxin standard curves

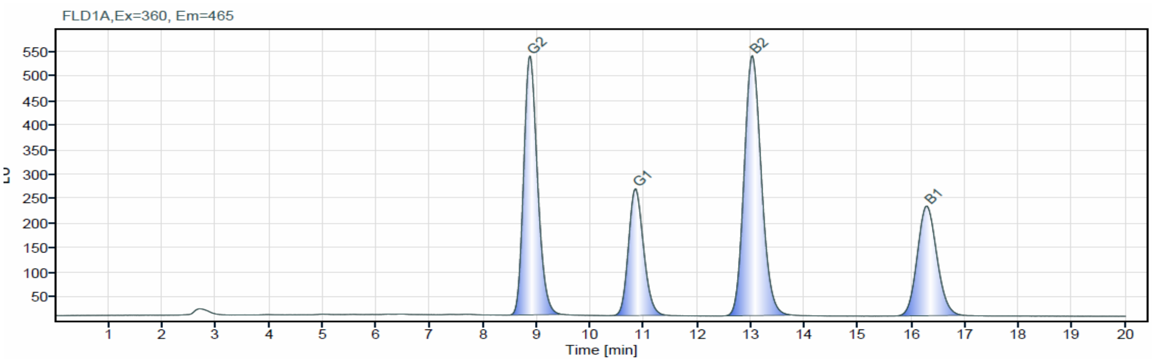

Figure 2. 2: Chromatogram of different aflatoxins from standard (10 µg/L for B_1 and G_1; 2.5 µg/L for B_2 and G_2

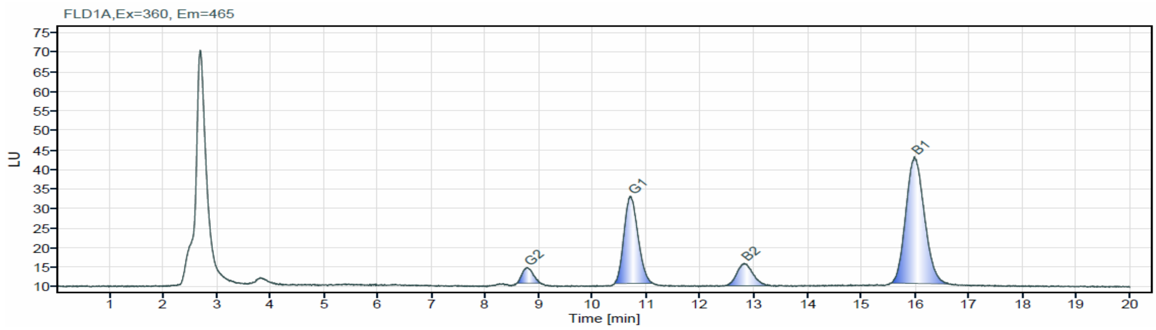

Figure 2. 3: Chromatogram of aflatoxin from cashew nuts sample

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.

2.4 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.

2.5 Results and Discussion

2.5.1 Aflatoxins contamination in cashew nuts

The results showed that aflatoxin B_1 was the most predominant toxin among the tested aflatoxins in both raw and roasted cashew nut samples (Table 2.4). The levels of contamination ranged from not detected (less than LOQ) to 3.29 µg/kg for both aflatoxin B_1 and total aflatoxins in the samples.

Table 2.3: Total and Aflatoxin B_1 (µg/kg) contamination (range) and frequency for total aflatoxin in Masasi and Newala districts in raw and roasted cashew nuts

District	Process	AFB_1	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

Key:
TAF: Total Aflatoxin,
LOQ: Limit of Quantification,

ND: Not Detected

All roasted cashew nuts from both districts had total aflatoxin less than 3 µg/kg, while more than 80% of raw cashew nut samples had total aflatoxins less than 3 µg/kg. Roasted cashew nut was less contaminated where by about 63% and 46 % of the samples from masasi and Newala respectively had total aflatoxins less than LOQ (0.16 µg/kg) while 25 % of raw cashew nuts samples from Masasi had total aflatoxins less than LOQ and none of raw cashew nuts samples from Newala had total aflatoxins less than LOQ. All the samples had total aflatoxins contamination below the recommended maximum residues of 4 µg/kg set by European Commission (2010) or 10 µg/kg set by FAO and WHO (1995) for similar products such as pistachio and almond. Although raw cashew nut had higher level of total aflatoxin as compared to roasted cashew nut samples.

2.5.1.1 Aflatoxins contamination within the districts

The levels of aflatoxins in cashew nuts (raw and roasted) are indicated in Table 2.4. Statistical difference did not exist between aflatoxin B_1 and total aflatoxins. Statistical difference was observed between roasted and raw cashew nut where by raw cashew nuts were found to have statistically higher levels of total aflatoxins and AFB_1 compared to roasted cashew nuts in both districts. 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 2.4: Aflatoxin contamination (µg/kg) in raw and roasted cashew nuts from Masasi and Newala districts

District	Process	AF G_2	AF G_1	AF B_2	AF B_1	Total
Masasi	Raw	0.05 ± 0.02^a	0.01 ± 0.02^a	0.02 ± 0.01^a	1.71 ± 0.43^a	1.79 ± 0.43^a
	Roasted	0.04 ± 0.01^a	0.01 ± 0.02^a	0.02 ± 0.01^a	0.83 ± 0.12^b	0.90 ± 0.12^b
Newala	Raw	0.04 ± 0.02^a	0.03 ± 0.02^a	0.03 ± 0.01^a	2.02 ± 0.23^a	2.12 ± 0.22^a

Roasted	0.04±0.01[a]	0.02±0.01[a]	0.03±0.01[a]	0.87±0.13[b]	0.97±0.14[b]

Means with different letters within the column are statistically significant.

The lower value of aflatoxins in roasted cashew nuts can be accounted by application of heat during roasting that can degrade the toxins, this was demonstrated by Martins *et al.* (2017) who investigated aflatoxins degradation during peanut roasting. It was reported that roasting at 160, 180 and 200 °C resulted in aflatoxins reduction of 61.6, 83.6 and 89.7%, respectively. Also Farahmandfar and Tirgarian, 2020 conducted a study on the effect of roasting conditions on the extent of the degradation of aflatoxins, it was reported that maximum reduction of aflatoxins B_1, B_2, G_1 and G_2 was 37.9%, 39.8%, 37.4% and 40.4%, respectively, occurred at the higher roasting times, temperatures and initial concentrations of the aflatoxins.

For those samples where aflatoxins were detectable, aflatoxin B_1 was generally the major contributor to total aflatoxins. The levels of aflatoxins in this study were found to be higher than the levels found in the studies done in Nigeria and South Africa (Adetunji *et al.,* 2019) that observed total aflatoxins in cashew nuts to range 0.03-0.77 µg/kg and 0.01-0.28 µg/kg, respectively. the low The absence or low value of mycotoxins in the cashew nuts from both countries could be due to a number of factors such as moisture content of the cashew nut ranging between 2.19 % to 2.76 % (Ubwa *et al*., 2014), also the nuts are embedded in a hard shell under which is very difficult for fungi to proliferate while in the field. High levels of aflatoxins in cashew nuts (31.50 µg/kg) have been detected in a study done in north eastern Brazil in 2010 (Milhome *et al*., 2014). The presence of aflatoxins in processed nuts could be explained by possible fungi growth during storage or shipment prior to processing.

Another study done in Vietnam on multi-mycotoxin (18 toxins) in cashew nuts showed a maximum level of contamination of up to 32.1 µg/kg for AFB_1, while total AF concentrations varied from 0.6 µg/kg to 39.6 µg/kg (Tran-Lam *et al.*, 2021). Nut samples originated from tropical countries accounted for a large proportion of the aflatoxin-positive sample because high humidity and warm temperature favours the growth of toxigenic molds, which holds true for both the tropical and sub-tropical regions (Bhat and Reddy, 2017).

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).

2.5.2 Demographic charateristics

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%) as shown in Table 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).

Table 2.5: Demographic, general information and awareness of aflatoxins in small scale cashew nuts dealers in Newala and Masasi districts

Category	Sub-category	Number of observation n (%)	Aflatoxin knowledge n (%)
Gender	Male	43(36)	11(9)
	Female	77(64)	22(18)
District	Newala	59	6(10)
	Masasi	61	12(20)
Education level	Primary	70(58)	4(6)
	Secondary	46(39)	13(28)
	University/tertiary	4(3)	(1)33
Type of respondent	Processors	12(10)	1(8)
	Consumer	48(40)	0(0)
	Both	60(50)	15(25)
Type of end product	Raw	2(3)	

Roasted	4(6)
Both raw and roasted	66(91)

Key: n is the total number of repondents

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

Very few respondents (<20%) in either category heard of aflatoxins in their lifetime as in Table 2.5. Almost 30% of all respondents who heard the word aflatoxins or “sumu kuvu” in the national language heard it during different trainings. For example, more than 50% of all respondent had primary school education but only 6 % heard the word aflatoxins whereas 28% of respondents who have secondary school education heard about aflatoxins. Similar findings in a study on the awareness of mycotoxins infections in Kilosa district of Tanzania reported 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). Also, in Malawi Matumba *et al.* (2015) reported that smallholder farmers lack of awareness on health effects caused by mould and mycotoxins.

In addition to the respondents who were aware on aflatoxins, all were also aware that (i) aflatoxins is caused by fungi (ii) cashew nuts can be contaminated by aflatoxins (iii) poor storage might be a cause of fungal growth and thus contaminated with aflatoxins, (iv) 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 remained 16 dealers mention that high moisture content during storage of cashew nuts can results to fungal growth. 66 % of the processors produce both roasted and raw cashew nuts (Table 2.5) but roasted cashew nuts are highly consumed than raw (more than 50%).

2.5.2.2 Storage practices of cashew nuts

The cashews are stored in the form of shelled 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 nut for not more than three month (Table 2.6). A study from Nigeria (Lawal and Fagbohun, 2014) about aflatoxin contamination and nutritive values of processed cashew (*Anacardium occidentale L*) nuts during storage reported an increase in toxigenic fungi after storing the dried cashewnut for five months.

Table 2.6: Storage information

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 backets	53	26

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 reported by Azam-Ali and Judge in 2001 showed an estimation of 280 000 households, covering an area of 400 000 hectares, are involved in cashew production and the government was actively supporting them in improving the farming activity by supplying the necessary agronomical items so as to maximize output (Azam-Ali and Judge, 2001).The processed cashew nuts were 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 cashew nut 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, some of the packages had some information on the label but others were lacking the important details such as date of manufacturing and expiry, storage condition and manufacturer address. Ramadhani *et al. (*2014) found similar result where by plastic buckets (87.5%) and other materials such as paper boxes (12.5%) were used but for street vendors plastic films (polyethylene bags) were the main packaging materials used (97.5%). A study that was conducted in Osun state in Nigeria to evaluate the effect of packaging materials on moisture and microbiological quality of packed roasted cashew nuts reported that plastic and glass bottles had counts within the acceptable limits (Oladapo, 2014).

2.6 Conclusion

Aflatoxicosis is still one of the main public health concerns in Tanzania that lead to health hazards in the population, but cashew nuts has very minimal contribution to the health hazard due to aflatoxin contamination since the study has shown very minimal aflatoxin contamination in cashew nuts. This study showed that roasted cashew nuts are less contaminated than raw cashew nuts and the reason might be the heat treatment during roasting which reduces aflatoxin concentration. The study revealed that stakeholders along cashew nuts value chain have low awareness to aflatoxin, which is an important aspect in the reduction or maintaining low contamination.

Moreover the storage time have an effect to the aflatoxin contamination, in this study most of the processors of the cashew nut were not storing for more than three month before selling which contributed to low level of aflatoxin.

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CHAPTER THREE

3.0 ESTIMATING HUMAN EXPOSURE TO AFLATOXIN THROUGH CONSUMPTION OF CONTAMINATED CASHEW NUTS: A CASE STUDY OF NEWALA AND MASASI DISTRICTS.

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3.1 Abstract

Human exposure to aflatoxin through consumption of contaminated cashew nuts in Masasi and Newala ditricts of Mtwara region, in Tanzania was estimated. Total aflatoxins (AFs) and aflatoxin B_1 (AFB_1) contamination of cashew nuts were determined from 60 samples. The samples were bought from small scale processors in two districts (Newala and Masasi) in February, 2021. Data on the rate of consumption was obtained through a structured questionnaire which involved 120 respondents; the data was used for the calculation of the Estimated Daily Intake (EDI) and the exposure assessment. The risk assessment for aflatoxins was evaluated by the qualitative Margin of Exposure (MOE) and the quantitative hepatocellular carcinoma (HCC) risk approach. Aflatoxins concentration ranged from not detected to 3.29 μg/ kg and to 3.24 μg/ kg for cashew nuts in Masasi and Newala, respectively. The estimated cancer risk (HCC) in this study ranged from 0.006 to 0.394 cancers per year per 10^5 individuals, upon consumption of cashew nuts with aflatoxin B_1 greater or equal to LOQ to highest contaminated samples respectively. The estimated daily intake (EDI) ranged from 0.23-14.08 μg/ kg bw and 0.21-13.89 μg/ kg bw for Masasi and Newala districts respectively. The results indicate that all levels of aflatoxins selected for calculation of health risk assessment had EDI value above the recommended/safe level (0.17 μg/kg bw) and the MOE above recommended level of $<10^5$ inspite of having AFB_1 concentration below legislation limits. Consumption of contaminated cashew nut samples increases the health risks concerns. This calls for implementation of mitigation measures to reduce to acceptable level or totally eliminate contamination.

Key words: Cashew nuts, aflatoxins, health risks, Margin of exposure (MOE), estimated daily intake (EDI)

3.2 Introduction

Dietary exposure to aflatoxins is considered a major public health concern, especially for subsistence farming communities in sub-Saharan Africa and South Asia, where dietary food crops are often highly contaminated with aflatoxins due to hot and humid climates and poor storage, together with low awareness of risks and lack of enforcement of regulatory limits (Gong *et al.*, 2016). High and moderate exposure of aflatoxins cause aflatoxicosis in humans and animals which can lead to haemorrhage, acute liver damage, edema, digestion problems and death (Sarma *et al.,* 2017). Chronic aflatoxicosis includes terratogenic effects associated with congenital malformation, may be an underlying cause of stunted child growth as well as lowering immunity (Sarma *et al.,* 2017). AFB_1 is the most toxic of the aflatoxins, it is a potent liver carcinogen which causes hepatocellular carcinoma (HCC) in humans and a wide variety of animal species (Wu *et al.,* 2014).

The International Agency for Research on Cancer (IARC) on several occasions and many experimental and human studies has proved that aflatoxins are group 1 human carcinogen (Wu *et al.,* 2014). Furthermore, the individuals who are exposed to aflatoxins and the hepatitis B virus (HBV) are common in developing countries which greatly increases HCC risk (Wogan *et al*., 2012).

A number of epidemiological studies have shown clear associations between aflatoxins exposure and incidence of hepatocellular carcinoma in areas with high prevalence of chronic hepatitis B, which is itself a risk factor for liver cancer (EFSA, 2007). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 64^{th} meeting in 2005 decided that compounds that are both genotoxic and carcinogenic should be based on estimated Margins of Exposure (MOEs). Also the Scientific Committee (SC) of the European Food Safety Authority (EFSA) recommends the application of the MOE approach as a harmonized methodology for assessing the risk of genotoxic and carcinogenic substances which may be found in food and feed, irrespective of their origin.

The total aflatoxins should not exceed 4 μg/ kg in groundnuts, nuts, dried fruits, cereals and processed products as per the European Food Safety Authority (EFSA, 2007). As for the Tanzania Standards (TZS 739:2010) for cashew nuts the concentration should not exceed 5 μg/kg for aflatoxin B_1 and 10 μg/kg for total aflatoxins. This study was therefore aimed at determining the health risks associated with consumption of contaminated cashew nuts- a case study of Newala and Masasi district.

3.3 Materials and Methods

3.3.1 Study Area

The study was conducted in Masasi and Newala districts in Mtwara region. The districts were picked because they are among the districts of major producers of the cashew nuts in Mtwara region (CBT, 2018). In addition the cashew nuts are the major cash crops in the districts. The study population was small scale processors of cashew nuts, workers in the cashew processing chain as well as cashew nuts sellers.

2.3.2 Sampling

The study employed probability sampling technique (Adwok, 2015). Processors were randomly selected in their respective wards through simple random sampling technique in which selection of processors based on their involvement in cashew nuts processing and in cashew nuts chain. Other information on the rate of consumption was collected from street vendors and people involved in the processing chain of cashew nuts.

2.3.3 Sample size

A total of 60 samples (Kothari and Garg, 2014) of which 40 roasted (24 samples from Newala and 16 from Masasi) and 20 raw samples (12 from Newala and 8 from Masasi) were collected. 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 200g zipped plastic bags and transported Tanzania Bureau of Standards food laboratory in Dar es Salaam for analysis.

2.3.4 Consumption data of cashew nut

Data of cashew nuts consumption were collected through a structured questionnaire as the exposure route was through ingestion. A total of 120 respondents were enterviwed on their cashew nut eating habits from the two districts Newala and Masasi. In this questionaire, the consumption data were collected based on the reference of 100 g zipped bag of cashew nut, which were used in the assessment. If the respondents were consuming approximately of one bag meant that his consumption is 100g, if the consumption is two bags then the consumption is 200 g of cashew nut in a day.

2.3.5 Exposure estimation

Calculation of the Estimated Daily Intake (EDI) was done by using the mean levels of aflatoxins obtained in cashew nut samples, the daily intakes of cashew nut (Adetunji *et al*., 2018) and the average body weight for Tanzanian adult which was estimated to weigh 70 kg (Kimanya *et al*., 2021). The EDI for mean aflatoxins was calculated according to the following formula and expressed in μg/kg of bodyweight/day (μg/kg b.w/day).

$$EDI\left(\frac{ng}{kg}\right)=\frac{Contamination\,level\left(\frac{ng}{g}\right)*Amount\ consumed\,(g)}{Body\ weight\,(kg)}$$

2.3.6 Risk characterization

Two international methods for risk characterization were evaluated in this study i.e. the qualitative Margin of Exposure (MOE) approach proposed by EFSA (Schrenk *et al*., 2020) and the quantitative Hepatocellular carcinoma (HCC) risk approach proposed by FAO/WHO (JECFA, 1998). Risk characterization for genotoxic and carcinogenic compounds such as aflatoxins was based on the margin of exposures (MOEs), which was calculated by dividing the Benchmark dose lower limit (BMDL) by EDI (EFSA, 2007).

$$MOE = \frac{BMDL_{10}}{EDI}$$

JECFA concluded that the $BMDL_{10}$ amounting to 170 ng/kg bw per day as the (Schrenk *et al*., 2020). The $BMDL_{10}$ previously derived by EFSA was based on data on the liver tumor incidences in rats (EFSA, 2007). If the MOE value is less than 10,000 or the EDI is greater than 0.17 µg/kg bw, the EDI is considered of concern from a public health point of view. In general, a smaller MOE corresponds to a greater concern (EFSA, 2005).

In hepatitis B surface antigen-positive individuals ($HBsAg^{+}$), the AFB_1 carcinogenic potency is estimated at 0.3 cancer cases/year/10^5 individuals per 1 ng/kg bw per day. In hepatitis B surface antigen-negative individuals ($HBsAg^{-}$), the AFB_1 carcinogenic potency is estimated at 0.01 cancer cases/year/10^5 individuals per 1 ng/kg bw per day (JECFA, 1998). To estimate the risk posed by dietary exposure to AFB_1, an excess risk model was simulated as follows:

$$Population\, risk = Exposure * Average\ potency$$

$$Average\ potency = 0.3 * P + 0.01 * (1 - P)$$

P represents the $HBsAg^{+}$ prevalence rate for all different age group of which in Tanzania was estimated to be 6.2% (Kilonzo *et al.*, 2018; Kimanya *et al*., 2021).

3.4 Statistical analysis

Statistical Package for Social Sciences (IBM SPSS® Version 25 (2017) was used for calculating frequencies and descriptive summaries on data for eating practices. Data on health risk assessment upon consumption contaminated cashew nuts were analyzed using R-version 4.0.3 (2020). Analysis of variance (ANOVA) was used to test for significant difference on health risk index in raw and roasted cashew nuts from different districts.

3.5 Results and Discussion

3.5.1 Cashew nut consumption estimates

Cashew nut being one of the main crops grown in the study area, it was shown that the consumption of it is daily (more than 50%) especially during high season. The estimated eating habit for cashew nut was performed where by in both districts most of the respondents were consuming average of 200g of cashew nut per day (Table 3.1). Most of the consumers in both districts prefer roasted cashew nut.

Table 3.1: Eating practices for cashew nuts dealers in Masasi and Newala

Category	Response	District	
		Newala	Masasi
Frequency	Rarely	7 (63.6)	4 (36.4)
	Sometimes	17 (48.6)	18 (51.4)
	Daily	35 (47.3)	39 (52.7)
Amount eaten (g)	100	15 (39.5)	23 (60.5)
	200	40 (60.6)	26 (39.4)
	More than 300	4 (25.0)	12 (75.0)
Type eaten	Raw	0 (0.0)	1 (100.0)
	Roasted	32 (45.7)	38 (54.3)
	Baked	7 (53.8)	6 (42.2
	Any type	20 (55.6)	16 (44.4)

Number in parenthesis are the percentages in each category

3.5.2 Exposure assessment

The EDI values were calculated based on the lowest to large portion size of consumption from respondents of both Masasi and Newala districts. The highest exposures to AFB_1 were obtained for people whose consumption level is estimated to be 300 g at maximum AFB_1 (Table 3.2). The results on exposure assessment showed that, all levels of aflatoxins selected for calculation had EDI value above the recommended/ safe level (0.17 µg/kg bw) (despite of having AFB1 concentration below legislation limits (safe limits) (Ding et al., 2015; Lien et al., 2019; Magoha et al., 2014; Shephard, 2008; Udovicki et al., 2021) . Adetunji et al. (2018) assessed the microbiological quality and risk assessment for aflatoxins in cashew nuts and groundnut consumers in Lagos and Ogun state, Nigeria. It was found that the risk of primary liver cancer for cashew nuts consumers was 0.01 cancer year−1100, 000−1 person, respectively. These findings were relatively lower as compared to the findings in this stud3.2) inspite of having concentration of AFB_1 below legislation limits (safe limits).

However, if non-detected samples (concentration <0.16 for AFB_1) was replaced with LOQ value of AFB_1 during health risk assessment, 33% of all samples, could have the EDI less than recommended level than if concentration of AFB_1 actual level was used in calculations of EDI. Since most agencies have not set a tolerable daily intake for AFs due to its genotoxic and carcinogenic nature (Cano-Sancho *et al.*, 2013**)** and if the Provisional Maximum Tolerable Daily Intake (PMTDI) of 1.0 ng of AFB_1/kg body weight (BW) for adults and children without hepatitis B virus infection as proposed by Kuiper-Goodman, (1998) was used, all samples with levels equal or less than LOQ could also have EDI less than PMTDI. Consumption of high amount of contaminated cashew nut samples increases the health risks concerns as shown in the Table 3.2. These findings are similar to (Kujbida and Maia, 2019) who found that the consumption of less contaminated peanut or cashew nut samples (0.35 ng AFB_1/g) displayed a daily intake from 0.35 to 0.88 fold lower than the PMTDI (1.0 ng of AFB_1/ kg body weight).

Table 3.2: Health risk (Hepatocellular carcinoma -HCC) assessment on consumption of cashew nuts produced in Masasi and Newala

			Raw				Roasted			
District	**Consumption (g)**	**AFB_1 level**	**AFB_1**	**EDI (µg/kg bw)**	**MOE**	**HCC**	**AFB_1**	**EDI (µg/kg bw)**	**MOE**	**HCC**
Masasi	300	Max	3.29	14.08	12.07	0.394	2.36	10.10	16.82	0.283
		Mean	1.79	7.68	22.12	0.215	0.90	3.84	44.23	0.108
		LOQ	0.16	0.69	247.92	0.019	0.15	0.64	264.44	0.018
	200	Max	3.29	9.39	18.11	0.263	2.36	6.74	25.24	0.188

		Mean	1.79	5.12	33.19	0.143	0.90	2.56	66.35	0.072
		LOQ	0.16	0.46	371.88	0.013	0.15	0.43	396.67	0.012
		Max	3.29	4.69	36.21	0.131	2.36	3.37	50.47	0.094
	100	Mean	1.79	2.56	66.37	0.072	0.90	1.28	132.70	0.036
		LOQ	0.16	0.23	743.75	0.006	0.15	0.21	793.33	0.006
		Max	3.24	13.89	12.24	0.389	3.24	13.89	12.24	0.389
	300	Mean	2.12	9.10	18.69	0.255	0.97	4.16	40.91	0.116
		LOQ	0.15	0.64	264.44	0.018	0.16	0.69	247.92	0.019
		Max	3.24	9.26	18.36	0.259	3.24	9.26	18.36	0.259
Newala	200	Mean	2.12	6.07	28.03	0.170	0.97	2.77	61.36	0.078
		LOQ	0.15	0.43	396.67	0.012	0.16	0.46	371.88	0.013
		Max	3.24	4.63	36.71	0.130	3.24	4.63	36.71	0.130
	100	Mean	2.12	3.03	56.06	0.085	0.97	1.39	122.72	0.039
		LOQ	0.15	0.21	793.33	0.006	0.16	0.23	743.75	0.006

Key: AFB_1: Aflatoxin B_1 concentration,
LOQ: Limit of quantification,
MOE: Margin of Exposure,
EDI: Estimated Daily intake
HCC: Hepatocellular carcinoma

3.5.3 Risk characterization

The MOE above recommended level of $<10^5$ (despite of having AFB1 concentration below legislation limits (safe limits) (Ding et al., 2015; Lien et al., 2019; Magoha et al., 2014; Shephard, 2008; Udovicki et al., 2021) . Adetunji et al. (2018) assessed the microbiological quality and risk assessment for aflatoxins in cashew nuts and groundnut consumers in Lagos and Ogun state, Nigeria. It was found that the risk of primary liver cancer for cashew nuts consumers was 0.01 cancer year−1100, 000−1 person, respectively. These findings were relatively lower as compared to the findings in this study inspite of having concentration of AFB_1 below legislation limits (safe limits). However, if non-detected samples (concentration <0.16 for AFB_1) was replaced with LOQ value of AFB_1 during health risk assessment, 33% of all samples, could have greater MOE value than if concentration of AFB_1 actual level was used in calculations of MOE.

The Cancer risk (HCC) in this study ranged from 0.006 to 0.394 cancers per year per 10^5 individuals, upon consumption of cashew nuts with aflatoxin B_1 greater or equal to LOQ to highest contaminated samples respectively. The HCC in this study was comparable to the one found in the study done in Brazil by Andrade *et al.*, 2013 and New Zealand by Cressey and Reeve (2013) but lower compared to different studies done in similar products in different African countries as reviewed by Shephard (2008). Different studies on health risk assessment in cashew nuts and similar products reported lower value of MOE ($<10^5$) despite of having AFB_1 concentration below legislation limits (safe limits) (Ding *et al.*, 2015; Lien *et al.,* 2019; Magoha *et al.,* 2014; Shephard, 2008; Udovicki *et al.,* 2021). Adetunji *et al.* (2018) assessed the microbiological quality and risk assessment for aflatoxins in cashew nuts and groundnut consumers in Lagos and Ogun state, Nigeria. It was found that the risk of primary liver cancer for cashew nuts consumers was 0.01 cancer year−1100, 000^{-1} person, respectively. These findings were relatively lower as compared to the findings in this study.

3.6 Conclusion

Based on the findings of the study, aflatoxins contamination in cashew nuts was very minimal because none of the samples had total aflatoxins contamination greater than the recommended maximum residues of 4 µg/kg set by European Commission or 10 µg/kg set by the Tanzania Standards. The EDI depends on the level of aflatoxin B_1 contamination and amount of cashew nuts consumed. The estimated Daily Intake for all levels of aflatoxins selected for calculation of health risk assessment was above the recommended/safe level and the Margin of Exposure (MOE) was above recommended level of $<10^5$. The Cancer risk (HCC) in this study increased with the increase of the level of aflatoxin B_1 and the amount of cashew nuts consumed. Therefore, long-term consumption of the aflatoxins contaminated nuts even if the level of contamination is lower than the recommended safe level it has carcinogenic and toxigenic effects on the human health.

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CHAPTER FOUR

4.0 GENERAL CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Aflatoxicosis is still one of the main public health concerns in Tanzania that lead to health hazards in the population, but cashew nuts has very minimal contribution to the health hazard due to aflatoxin contamination since the study has shown very minimal aflatoxin contamination in cashew nuts. This study showed that roasted cashew nuts are less contaminated than raw cashew nuts and the reason might be the heat treatment during roasting which reduces aflatoxin concentration. The study revealed that stakeholders along cashew nuts value chain have low awareness to aflatoxin, which is an important aspect in the reduction or maintaining low contamination.

Also the study revealed that the estimated daily intake for all levels of aflatoxins selected for calculation of health risk assessment was above the recommended/safe level and the Margin of Exposure (MOE) was above recommended level of $<10^5$. This shows that for aflatoxin contamination even if the contamination level is very minimal, it is not safe. The Cancer risk (HCC) in this study increased with the increase of the level of aflatoxin B_1 and the amount of cashew nuts consumed. Therefore, long-term consumption of the aflatoxins contaminated nuts even if the level of contamination is lower than the recommended safe level it has carcinogenic and toxigenic effects on the human health.

4.2 Recommendations

Programs for monitoring the level of aflatoxins in cashew nut should be implemented continuously to ensure that the contamination level remain to be low or eliminated because cashew nut is one of the important cash crops in Tanzania.

In order to reduce the contamination at lower level or eliminate strict hygienic measures should also be implemented during storage, drying and packing which will enhance food safety, international trade efforts and improved public health. Also there is a need to educate people on the impact of aflatoxins because awareness on aflatoxins was very minimal especially to the processors who had primary school education.

In addition, proper packaging materials and good storage practices should be enforced. It is recommended that establishment of a systematic food control systems such as Good Manufacturing Procedure (GAP) and Hazard Analysis Critical Control Point (HACCP) will minimize the contamination.

More studies should be conducted on the investigation of microbiological quality of cashew nut available in the market because of the nature of processing by small scale processors is done by using bare hands.

APPENDECES

Appendix 1: Questionnaire to evaluate factors associated with aflatoxins contamination of cashew nuts

This questionnaire is aimed to cashew nuts processors and retailers on factors and practices associated with mycotoxins contamination in cashew nuts.

PART A: GEOGRAPHICAL LOCATION

1. Region……………………………………………………………
2. District……………………………………………………………
3. Division…………………………………………………………….
4. Ward……………………………………………………………
5. Village……………………………………………………………

PART B: RESPONDENT DETAILS

6. Date of interview: Day……Month………Year…..………
7. Sex of respondent: ☐ Male ☐ Female
8. Age of the respondent (years)……...............
9. Level of education of the respondent
   - ☐ Primary education level
   - ☐ Secondary school education level
   - ☐ College/University education
   - ☐ Informal education
   - ☐ Not attended any school

10. What type of cashew nut is your end product?

☐ Raw ☐ Roasted

☐ Any other (specify)

…………………………………………………………

PART C: KNOWLEDGE ON MYCOTOXINS

11. Have you ever heard about mycotoxins?

☐ Yes ☐ No

12. If yes, where did you first hear about it?

☐ Radio ☐ TV ☐ Hospital

☐ Training ☐ Others (Specify)……………

13. Do you know aflatoxins can contaminate cashew nuts?

☐ Yes ☐ No

14. What factors contribute to aflatoxins contamination in cashew nuts?

☐ Rodents/Insects/Molds ☐ Do not know

☐ Poor harvesting ☐ Poor storage conditions

☐ Post and pre harvesting factors ☐ Others (Specify) ……………

15. How do you store the processed cashew nuts?

☐ Godown room ☐ Kihenge ☐ On the floor

☐ Other ways……………………..

16. Do you know consumption of cashew nuts contaminated with aflatoxins can cause health effect to human?

☐ Yes ☐ No

☐ Don't no

PART D: CASHEW NUTS HANDLING PRACTICES

17. How many bags/kg of cashew nut do you store? …... …...........

18. How long do you store cashew nut before selling? …………… (months)

19. Where do the raw material come from?

☐ Yes AMCOS ☐ Own farm ☐ others……………

20. How do you keep raw and processed cashew nuts?

☐ Bare ground ☐ Raised platforms

☐ Jute/Sisal bags ☐ Plastic/synthetic bags

☐ Others (Please specify) ……………………………..

21. Do you carry any inspection of cashew nuts before processing?

☐ Yes ☐ No

22. If, yes what parameters do you check

☐ Color ☐ Moldy smell

☐ Mycotoxins ☐ Any other (specify)…………………

PART E: RISK ASSESSMENT CHECKLIST

No.	**Question**	**Response options**	**Answer**
1.	How many are you in your family?	1 = less than 4 2 = above 4	
2.	Do you eat cashew nuts?	1 = Rarely 2 = Sometimes 3 = Often	
2a.	How often do you consume cashew nuts?	1= never 2 = 2- 4 times a week	

		3= 2- 4 times a week 4= daily	
3.	How much do you consume per serving?	1= 100g 2=200 g 3= more than 300 g	
4.	Type of cashew nuts consumed	1 = Raw 2 = roasted	
5.	Where do you get cashew nuts?	1 = Local market 2 = own farm	

Appendix 2: Data analysis report outputs

Anova for G_1

```
            Df  Sum Sq  Mean Sq F value Pr(>F)
District     1 0.00500 0.005001   2.362  0.130
Process      1 0.00065 0.000650   0.307  0.582
Residuals   57 0.12071 0.002118
```

Anova G_2

```
            Df Sum Sq  Mean Sq F value Pr(>F)
District     1 0.0006 0.000624   0.067  0.796
Process      1 0.0006 0.000598   0.064  0.800
Residuals   57 0.5290 0.009280
```

Anova $\mathbf{B_2}$

```
            Df  Sum Sq  Mean Sq F value Pr(>F)
District     1 0.00960 0.009596   2.491  0.120
Process      1 0.00005 0.000047   0.012  0.913
Residuals   57 0.21956 0.003852
```

Anova B_1

```
            Df Sum Sq Mean Sq F value   Pr(>F)
District     1   1.02    1.02   0.482     0.49
Process      1  58.02   58.02  27.333 2.54e-06 ***
Residuals   57 120.99    2.12
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
>
```

```
$groups
              B1 groups
Raw     3.800035      a
Roasted 1.714085      b
```

Anova total

```
            Df Sum Sq Mean Sq F value   Pr(>F)    
District     1   1.45    1.45   0.662    0.419    
Process      1  58.85   58.85  26.875 2.98e-06 ***
Residuals   57 124.82    2.19                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
$groups
           Total groups
Raw     3.981913      a
Roasted 1.880978      b
```