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Determining aflatoxins contamination in locally processed peanut butter using fluorometry and hplc in Arusha city, Tanzania

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DETERMINING AFLATOXINS CONTAMINATION IN LOCALLY PROCESSED PEANUT BUTTER USING FLUOROMETRY AND HPLC IN ARUSHA CITY, TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Masters in Life Science and Bioengineering of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Food poisoning with mycotoxins produced by fungi is a global food safety issue. The consumption of mycotoxin-contaminated foods is responsible for several foodborne diseases outbreak worldwide**.** While peanuts are an important crop with both economic and nutritional significance in tropical and subtropical regions, their vulnerability to aflatoxin contamination, for example, makes them unsuitable for human consumption. This study aimed at assessing the aflatoxin contamination of locally processed peanut butter in Arusha city. Fifty samples of peanut butter from ten different firms (5 different batches per firm) were purchased at the local markets, supermarkets, and retail shops in the city. High-Performance Liquid Chromatography and Vicam fluorometer methods were used for aflatoxin analysis in the samples. Whereas the total amount of aflatoxins detected by Vicam fluorometer ranged from 5.6 to 720 ppb (µg/kg), by High-Performance Liquid Chromatography it ranged from 1 to 1981.37 µg/kg. These results showed a moderate positive correlation or relationship between High-Performance Liquid Chromatography and Vicam Fluorometer methods with r = 0.47. This implies that Vicam fluorometer can be more effective in initial determination of aflatoxins prior to food processing. The results further suggest that unhygienic processing practices, poor quality raw materials used for food processing, and the lack of quality control in local production chains might be the source of high aflatoxins contamination in peanut butter.

Accordingly, a moderate positive correlation in total aflatoxin concentration was revealed between High-Performance Liquid Chromatography and Fluorimeter methods (r = 0.47). This indicates that Fluorimeter could be a suitable and cost-effective pre-screening tool of aflatoxin levels in locally processed food products. It is recommended that farmers and food processors in local production chains can use Vicam fluorometer to determine the quality of raw materials before processing foods intended for human consumption. This could help farmers and food processors to timely identify aflatoxin contamination of their raw materials and products at pre and post-processing stages, and hence, improving the quality and safety and product market value.

DECLARATION

I, Mariamu C. Maarufu do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

..... 11/03/2019

Mariamu C. Maarufu **Date**

The above declaration is confirmed

..... 11th March 2019

Dr. Neema Kassim **Date**

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CERTIFICATION

The undersigned certifies that she has read the dissertation entitled, "**Determining aflatoxins contamination in locally processed peanut butter using Fluorometry and HPLC in Arusha City, Tanzania**" recommend for examination in fulfilment of the requirements for the degree of Master of Life Science and Bio-engineering of the Nelson Mandela African Institution of Science and Technology.

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Dr. Neema Kassim

11th March 2019

Date

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DEDICATION

I would like to dedicate this work to my loving husband, Mr. Andrea Daudi, my loving son, Joshua and my daughter, Sarah as well as my parents, Mr. and Mrs Hemed Maarufu, for their love, patience, support, and encouragement, during my studies.

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LIST OF ABBREVIATION AND SYMBOLS

n	Number of samples
µg/kg	Microgram Per Kilogram
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
CAC	Codex Alimentarius Commission
DNA	Deoxyribose Nucleic Acid
ITDG	Intermediate Technology Development Group
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
ppb	Parts per billion
RSD	Relative standard deviation
SE	Standard Error
SMEs	Small and Medium Enterprises
TLC	Thin Layer Chromatography
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background

Mycotoxins are natural fungal metabolites that have pathogenic roles and cause intoxication of foods worldwide (Ostadrahimi *et al*., 2014) while being estimated to contaminate 25% of the food produce worldwide (Kabak, 2010). The contamination of agricultural produce (mainly cereals, oily seeds and nuts such as peanut) with mycotoxins producing fungal compromises the safety of food and poses a serious health risk to consumers (Gong *et al*., 2002; Lewis *et al*., 2005). They induce toxic reaction upon consumption to humans and animals (Pena, 2010). Following the realization of mycotoxins contamination in foods, extensive research has been conducted on the nature of mycotoxins modes of action, effects to human health, and methods of their determination (Shephard, 2008).

Currently, more than 100 moulds have been reported to produce mycotoxins of around 400 secondary metabolites which are potential toxins (Kabak *et al*., 2006). Their contamination in foods differs depending on the climate such that the dry and hot conditions favour contamination during crop development, while the warm and wet conditions favour contamination after crop maturation (Cotty and Jaime-Garcia, 2007). Hot humid conditions, for example, favouring *aspergillus* growth, thereby leading to toxin production (Cotty and Jaime-Garcia, 2007). Fungi producing mycotoxins belong to the genera *Aspergillus*, *Penicillium,* and *Fusarium,* the mycotoxins that cause public health concern include aflatoxins, zearalenone, ochratoxin A, fumonisins and trichothecenes.

Among the mycotoxins, aflatoxin is the most potent foodborne food contaminant, found mostly in staple foods in many developing countries and has been reported as the most potent (Bankole *et al*., 2006; Ndung'u *et al*., 2013; WHO, 2006). It is produced by fungi before and after the harvest, with *Aspergillus flavus* and *A. parasiticus* being the most common species that produce aflatoxins (Agag, 2004; Elshafie *et al*., 2011; Walke *et al*., 2014). During a drought season, contamination may be severe in temperate regions (Cotty and Jaime-Garcia, 2007). The most common types are aflatoxin B1, B2, G1, and G2, with aflatoxin B_1 being the most potent (Golli-Bennour *et al*., 2010). *Aspergillus flavus* is the main source of B aflatoxins, while *Aspergillus parasiticus* produces both B and G types (Mutegi *et al*., 2012).

Worldwide, the major health risk associated with the consumption of aflatoxins contaminated foods is hepatocellular carcinoma which leads to approximately 550 000 to 600 000 new cases annually (Liu and Wu, 2010). Among other effects, the consumption of highly contaminated products has resulted in the outbreak of aflatoxicosis in Eastern African region in some countries like Somalia in 1997/98 and Kenya in 1982, 2001, 2004, and 2005 (WHO, 2006). Equally, in 2016, a total of 65 cases of the outbreak were reported in Chamwino, Kiteto, Chemba, Dodoma, and Kondoa districts in Tanzania. This incidence resulted in 17 deaths, while 48 people were confined at the Dodoma Regional Hospital. Accordingly, aflatoxin exposure has been associated with child growth retardation and malnutrition problems such as kwashiorkor and vitamin A deficiency due to nutritional interference. Aflatoxin, for instance, is said to bind the DNA leading to the decrease in protein synthesis and interfere with vitamin A metabolism in the body. Also, aflatoxins are known for their ability to lower the immunity, thereby creating a room for virus replication (Gong *et al*., 2002; Kamika and Takoy, 2011; Kimanya *et al*., 2008; Shirima *et al*., 2015; Turner *et al*., 2003; Unnevehr and Grace, 2013; Williams *et al*., 2004).

High levels of aflatoxins have been reported in peanuts and cereal grains such as maize (Kamika and Takoy, 2011; Kimanya *et al*., 2008). Peanuts are important crops of economic and nutritional significance in tropical regions (Guo *et al*., 2009). As an important crop, groundnut (*Arachis hypogaea L*) is among the dominant crops in Tanzania, grown by smallholder farmers as a source of both foods and incomes (Monyo *et al*., 2009). Usually, the crop is intercropped with cassava or cereals in the field under rainfed conditions. Eleven groundnuts varieties of different yielding capacities exist, with Pendo and Johari being identified as the highest yielding varieties (Buyecheki *et al*., 2010). Groundnuts, for instance, are categorised as a 3rd source of edible oil after sunflower and cotton seeds (Sibuga *et al*., 1992). Also, peanuts referred to as groundnuts contain various macronutrients and micronutrients that are beneficial to the human health. Apart from containing a high protein content, carbohydrates, fat, and vitamin A and E, peanuts also act as an important source of minerals such as folate, magnesium, zinc, iron, calcium, and dietary fibre (Briend and Weise, 2009). Furthermore, peanuts consumption is useful in weight management and reduction of chronic diseases risks such as heart diseases and diabetes (Griel *et al*., 2004). In that way, Peanuts, for example, are consumed as roasted, boiled or processed into peanut butter and oil (Afolabi *et al*., 2015; Chang *et al*., 2013). Also, in some communities, peanuts are used in food preparation as an ingredient or in the placement of oil. Most importantly, peanuts are

milled in combination with cereals like maize and millet in preparation of composite flour or complementary food. Equally, peanuts are sometimes consumed raw especially during harvesting, de-shelling, and packaging. Moreover, the consumption of raw peanut is believed to enhance sexual stamina, especially in men.

Despite the health benefits of peanuts, their vulnerability to fungal contamination (Mutegi *et al*., 2013) poses a great challenge on food quality and safety with a subsequent health risk to consumers (Mutegi *et al*., 2013; Ostadrahimi *et al*., 2014). The risk of aflatoxins contamination in peanuts increases during postharvest and is associated with poor handling and storage practices (Kaaya *et al*., 2006). The risk is high in sub-Saharan Africa where the conditions favour fungal growth (Wu and Khlangwiset, 2010). In tropical countries, for example, aflatoxin contamination of groundnuts and their products has been reported (Bankole *et al*., 2006; Mutegi *et al*., 2009; Soler *et al*., 2010; Ndung'u *et al*., 2013) as being at high levels in peanuts (Kamika and Takoy, 2011; Kimanya *et al*., 2008). Fungal infestation to peanuts can occur at various points of the food chain, including in the field, on storage, processing, and on the market (Mutegi *et al*., 2012). Peanut butter consumption has been related to foodborne illness and cause for outbreaks. (Chang *et al*., 2013).

The quality of peanut butter depends on practices from farm production throughout the processing stages, including shelling, storage, and product manufacturing. The choice of raw materials is very crucial during peanut butter processing as it contributes to the quality and safety of the final product. The peanuts to be used should be mature and dry, having uniform size, and free from fungal contamination (ITDG, 2002).

Over the past decades, various methods have been reported to be useful for determining mycotoxins in foods to ascertain both the exposure risks and toxic effects to humans and animals. Some methods of aflatoxin analysis including chromatographic methods like HPLC and TLC have been used to detect mycotoxins in foods (Barug *et al*., 2006). In contrast, immunologically-based methods such as ELISA, fluorescence polarization, fluorometry, strip, and biosensors have been used for fast screening (Pascale, 2009; Rahmani *et al*., 2009; Shephard, 2008; Trucksess *et al*., 2006). Notwithstanding its high operating costs and expertise to operate, HPLC is the most preferred method due to its good sensitivity and high accuracy. However, fluorometric method tends to mimic HPLC sample preparations with a reduced time of analysis, relatively less expensive, and an increased through put (Barug *et al*., 2006; Hoeltz *et al*., 2010). Also, the sample prepared for the analysis by fluorometer could be

further analysed by HPLC. In this study, the aim was to determine the levels of aflatoxin contamination of locally processed peanut butter using both fluorometer and HPLC methods.

1.2 Problem statement and justification

Currently, the country is dominated by small and medium scale food producers including peanut butter processors due to the new government policy that emphasises the increase in industrial production. Arusha is one among the regions having peanut butter processors and according to SEED initiative (2014), Arusha women entrepreneur enterprises employ women and train them on the production and marketing of peanut butter free from aflatoxins. This has led to the increase in consumption and marketing of locally pre-packed processed food products. Yet, there is limited data on the safety of these peanut butter products locally produced in Tanzania.

Nonetheless, most of these processors are not registered by the food quality and safety control agencies in the country (Chijoriga, 2017), thereby precipitating the need for the continued assessment of the aflatoxins contamination in peanut butter. This is particularly the case because the expansion of local food processing sector depends on the support from other related sectors such as scientific research sector that would provide support in pre and post-harvest technologies for quality insurance (ITDG, 2002).

In addition, limited quality control measures in the food chain, the lack of mycotoxins awareness, improper food handling and preservations, inconsistent product quality, as well as the lack of suitable equipment and technology increase the risk of aflatoxin contamination in peanut butter products (Mukantwali, 2014; Wagacha and Muthomi, 2008). The absence of reliable tools for screening of aflatoxin at local food processing units, for example, makes it difficult for producers and food processors to determine the aflatoxin status of their raw materials and the end products. As a result, aflatoxin levels in locally processed food products do not comply with the allowable national maximum limit of 5µg/kg for aflatoxin B1 and 10µg/kg in foods (Kimanya *et al*., 2008).

The assessment of aflatoxin contamination in these locally processed product is very important so as to assure product safety for human consumption and therefore, this study aimed at determining the levels of aflatoxins contamination using both Fluorometer and HPLC methods in locally processed peanut butter in Arusha city.

1.3 Objectives

1.3.1 General objective

This study aimed to determine the levels of aflatoxins contamination using both Fluorometer and HPLC methods in locally processed peanut butter in order to improve its quality and safety for human consumption in Arusha city.

1.3.2 Specific objective

(i) To determine the aflatoxins levels in locally processed peanut butter using HPLC method.

(ii) To determine the aflatoxins levels in locally processed peanut butter using fluorometer.

(iii) Comparing the efficiency of fluorometry method against HPLC method in detection of aflatoxins in peanut butter.

1.4 Research hypothesis

(i) Locally processed peanut butter is contaminated with high levels of aflatoxins that affect product safety and consumer health.

(ii) Fluorometry can be used as a cheap and reliable tool for aflatoxins determination in local peanut butter along the processing chain to ensure production of peanut butter free from aflatoxins.

1.5 Significance of the study

This study provides useful information to the local food safety control authorities such as Tanzania food and drug authority (TFDA) and Tanzania Bureau of Standards (TBS) about the aflatoxin contamination status of locally peanut butter products. Consequently, this helps in improving food safety system which, in turn, contributes to the protection and promotion of public health in Tanzania. Also, the information obtained in this study informs food producers and processors on the need to detect aflatoxins in foods during the pre-harvest and post-harvest practices in order to prevent aflatoxin contamination along the food value chain.

CHAPTER TWO

LITERATURE REVIEW

2.1 Groundnut production overview

Groundnuts are an important crop grown in many countries in the world, with India, USA, Sudan, China and Senegal being the major growing countries (Taru *et al*., 2010). The crop belongs to the Leguminosae family originated from Latin America and Portugal (Abalu and Etuk, 1986). Its production is mainly categorised as 13th main food crop, 3rd most vegetable source of protein, and 4th most edible oil source in the world, with 50% of people in the world using it for extraction of oil, 37% using it in confectionery, and 12% using it as seeds (Taru *et al*., 2010). More importantly, the groundnuts are said to contain 20-50% of protein, 10-20% of carbohydrates and 40-50% of fats (Sorrensen *et al*., 2004).

2.2 Groundnut production in Tanzania

Tanzania is among the major groundnut producing countries in the East and Southern Africa region (Monyo *et al*., 2009). Groundnut is one of the dominant crops grown by smallholder women farmers, both as a food and cash crop. Dodoma, Singida, Tabora, Mtwara, Shinyanga, Kigoma, and Mwanza are among the groundnuts producing regions in Tanzania (Bucheyeki *et al*., 2010; Mwenda *et al*., 1985; Monyo *et al*., 2009). Annually, the contribution of groundnut production to women growers is 6.3% of the overall mean income (Katundu *et al*., 2012). However, the vulnerability of groundnut to fungal attack from pre to post-harvest period poses a great challenge to its production and trade (Mutegi *et al*., 2009; Soler *et al*., 2010). This contributes to the loss of quality of the products and the decline in market value. During the 1970s, for example, Tanzania was among the groundnuts exporters to the other countries, which served as a source of export earnings. But after mycotoxins discovery, exportation declined due to aflatoxin standards set by the importing countries (Monyo *et al*., 2009).

2.3 Mycotoxins

Mycotoxins are the metabolites produced by the fungus. Their contamination in food and feeds has health effects on both humans and animals. Over 200 family members of *Aspergillus* moulds have been identified and some of them have been reported to produce mycotoxins (Pena, 2010). Some of the fungal strains have similar metabolic activities and growth speed but they produce a different quantity of mycotoxins and one or more forms of

metabolites. When the active growth phase ends on food substrate and the favourable conditions present, secondary metabolites favouring the survival of fungi can be produced by moulds (Santacroce *et al*., 2008). The group of food-borne mycotoxins includes deoxynivalenol, zearalenone, ochratoxins, fumonisins, and aflatoxins (Tola and Kabede, 2016). However, among these mycotoxins produced, aflatoxin is the most potent (Nageshe, 2018). The impacts of mycotoxins includes health effects to humans and animals, increase costs for health and veterinary care, waste of contaminated food and feed, reduce livestock production and costs/ use of resources for research, regulations and applications done to alleviate the severity of mycotoxin (Hussein and Brasel, 2001). Unfortunately, globally about 25% of the harvested crops are estimated to be contaminated with mycotoxins resulting in food loss for agriculture and industrial sectors (Martin *et al*., 2013). Due to the burden of mycotoxins problem, nationally and internationally the governmental health authorities have been adopting regulations and guidelines in addressing the problem for mycotoxins control in food and feeds (Bennett and Klich, 2003). Limits for mycotoxins presence in foods and feeds have been established by many countries in the world (Moretti *et al*., 2017).

2.4 Aflatoxin history

Aflatoxin was discovered in 1960s after the death of more than 100 000 young turkeys in England, whereby the disease was named as a Turkey disease. A survey on the outbreak was conducted and found that the disease was related to the consumption of ground meal from Brazil. In other studies that followed, the toxic nature of groundnut meal was revealed after producing typical symptoms related to those of Turkey disease (Blount, 1961; Richard, 2008; Wogan, 1966). It was discovered that the nature of toxin was originated from fungus *aspergillus flavus*, which made the disease to be named as aflatoxin. This event gave rise to the scientific interest, and hence, mycotoxicology research. Aflatoxin research led to further investigations into the mycotoxins from which other mycotoxins produced, including fumonisins, patulin, and ochratoxin were discovered (Bennett, 2010; Richard, 2008).

2.5 Aflatoxins

The aflatoxins production by *Aspergillus* species is associated with spore production. Among the variety of species, *Aspergillus flavus* and *A. parasiticus* are the most common that produce aflatoxins before harvest and during storage. A field fungus requires 22 - 25% of moisture while storage fungi require 13 - 18% of moisture (Agag, 2004). *Aspergillus flavus* strains differ in aflatoxin capabilities from nontoxic to highly toxic and are the main source of

B1 than G1 aflatoxins, whereas *Aspergillus parasiticus* strains differ less in their toxigenicity and produce aflatoxin B and G (Mutegi *et al*., 2012). *Aspegillus flavus* is more aggressive and more dominant in all commodities than *aspegillus flavus* since they adapt to a broad range of habitats and temperature, alongside being most abundant in the subtropical and warm atmosphere (Payne, 1998). Presence of water facilitates fungal germination, while temperature controls their growth rate (Pena, 2010).

Water activity of 0.70 and below affects both germination and mycelia growth, while 0.82 water activity favours toxin production. A temperature of 12 to 41^{o}C is suitable and favourable for aflatoxin production (Pena, 2010). Though *aspergillus flavus* and *parasiticus* are considered to be weak parasites, favourable conditions influence their colonisation in plant tissue and seeds. The percentage of infection on seeds can be low even in serious fungal contamination. This is because aflatoxin levels can be highly produced in individual seeds and so, even the infection on a few seeds can be of health and economic importance (Payne, 1998).

2.6 Biology of aflatoxigenic fungi

From an agronomic point of view, *aspergillus flavus* and *aspergillus parasiticus* are the plant pathogens, while ecologically, they are soil borne fungi growing on a variety of substrates such as living tissue (Payne, 1998; Wogan, 1966). Their population in the soil depends on their competition with microflora present in the soil. Soil temperature and moisture are the main factors that influence *aspergillus flavus* and *aspergillus parasiticus* population in the soil (Payne, 1998). They grow at 12 to 48^{0}C temperature, with an optimum growth temperature of 25 to 42^{0}C, and water potential below 35MPa. They are more competitive under high temperature and low water activity, which makes them dominant species in the soil. The amount and proportion of aflatoxins production depend on the substrate, mouldy strains, maturity, moisture, temperature and microbial interaction present (Goldblatt, 1969). But usually, B1 is present in a higher amount than B2 and G2 (Wogan, 1966).

2.7 Chemical structure of Aflatoxins

2.7.1 Aflatoxin B1 and B2

Under ultraviolet light, aflatoxins B1 and B2 emits blue fluorescent lights. The molecular formula for aflatoxin B1 is $C_{17}H_{12}O_6$, with a molecular weight of 312 g/mol, and melting point of 268 – 269°C, while for aflatoxin B2 is $C_{17}H_{14}O_6$, with a molecular weight of 314g/mol, and melting point of 286-289°C (Wogan, 1966). Aflatoxin B1 is the most toxic one with cytotoxicity, carcinogenic, and genotoxicity effects (Golli-Bennour *et al*., 2010).

2.7.2 Aflatoxin G1 and G2

Aflatoxins G1 and G2 emit yellow-green fluorescence under ultraviolet light. Their molecular formulae are $C_{17}H_{12}O_7$, with a molecular weight of 328 g/mol, and melting of 244 - 246 °C for aflatoxin G1 as well as $C_{17}H_{14}O_7$, with a molecular weight of 330 g/mol, and melting point of 237 - 240 °C for aflatoxin G2 (Wogan, 1966).

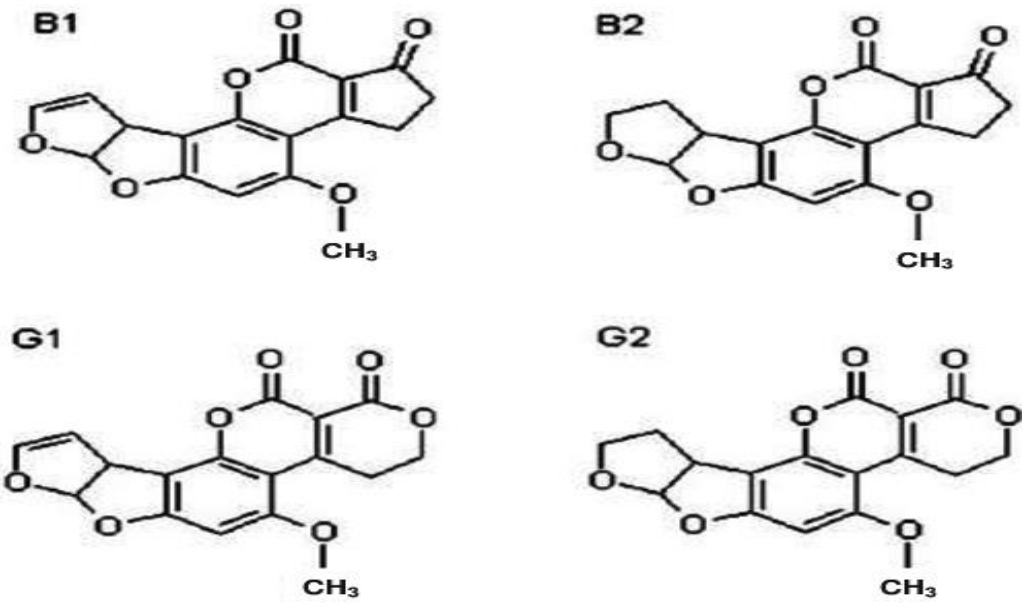


Figure 1: Chemical structure of aflatoxin B1, B2, G1 and G2 (Martins *et al*., 2013)

2.8 Effects of Aflatoxins

There is an increased concern on myocotoxins contamination in the food system. Aflatoxins contamination of food has great implication for human health. The effects of foodborne mycotoxins can be acute in high doses and its symptoms appear quickly or can be chronic for

a longer period as well as becoming lethal depending on dosage and exposure level (Hussein and Brasel, 2001). Significantly, growth impairment, cancer, and immune suppression are the health problems associated with mycotoxins in humans. Epidemiological studies have reported the association of aflatoxin consumption with liver cancer incidence (IARC, 2002). In Tanzania particularly, young children are being exposed to the early stages of life through the consumption of contaminated foods (Shirima *et al*., 2015). Previous studies showed that 99% of the children were exposed to aflatoxins and their growth retardation was associated with high blood aflatoxin-albumin adducts due to high frequencies of consumption of contaminated groundnuts (Egal *et al.*, 2005). Apart from human health aflatoxins contamination also has serious effects on food security and economic loss of food commodities like corn, peanuts, cottonseeds, and wheat (Tola and Kabede, 2016). Globally, due to aflatoxin contamination, it has been estimated that about US$ 1.2 billion is lost annually, with African economies losing US$ 450 million per year (IITA, 2010).

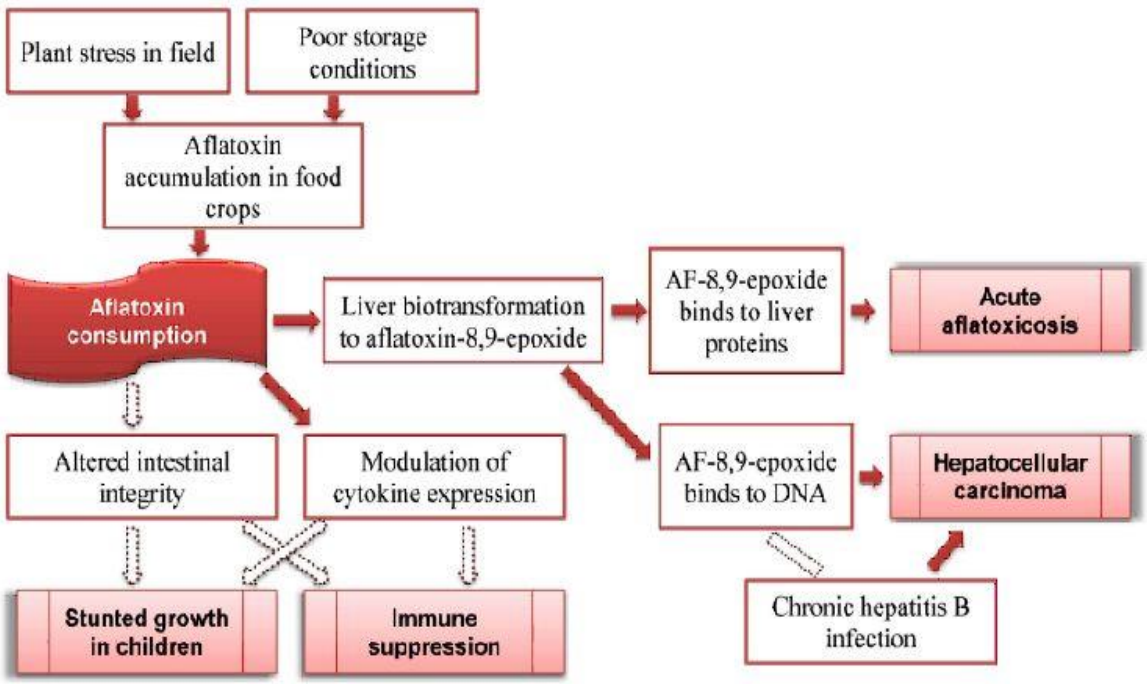


Figure 2: Aflatoxin and disease in humans (Negash, 2018).

2.9 Mechanisms of aflatoxins action

Upon consumption of contaminated food, aflatoxins are absorbed in the gastrointestinal tract and metabolized in the liver to form hydroxylated metabolites. These are oxidized to reactive epoxide resulting in the mutagenicity and carcinogenicity of aflatoxins B1, G1 and M1after its binding to macromolecules such as DNA and proteins (Pena, 2010). The unbound ones

which are excreted in the bile, urine, and into human breast milk increase the risks of exposure to the breastfed children (Agag, 2004; Pena, 2010).

2.10 Peanuts and aflatoxins

Peanuts are used for both human and animal feedstuffs. They are important in agricultural sector due to their various purposes, including the production of oil (Afolabi *et al*., 2015). Groundnuts are a good source of inexpensive nutritious food used in diets of rural people (Bankole *et al*., 2006). Despite their usefulness, *Aspergillus flavus* and *Aspergillus parasiticus* are the most common fungal species attacking peanuts (Mutegi *et al*., 2012). A high nutritional content of peanuts comprising protein, carbohydrates, fat, oil, and minerals makes them a good substrate for fungal growth and aflatoxin production (Barberis *et al*., 2012). The vulnerability of peanuts to aflatoxin contamination produced by fungi has been noted in different places (Guo *et al*., 2009) and high aflatoxin levels have been reported in peanuts and peanut products (Ding *et al*., 2012; Mphande, 2004; Soler *et al*., 2010). In Congo, for example, 70% of peanut samples contained aflatoxin levels that exceed the WHO maximum level (5µg/kg) for aflatoxins in food (Kamika and Takoy, 2011). The same was reported in Sudan where the traditionally prepared peanut butter samples had the highest level of aflatoxin B1 above the internationally regulated acceptance limit of 5–20 ppb (Elshafie *et al*., 2011). Moreover, a study conducted in Zimbabwe showed that peanut samples were contaminated with total aflatoxins to a high limit of 622.1 ng/g exceeding the regulatory aflatoxin limit of 15 µg/kg of all foods in Zimbabwe (Mupunga *et al*., 2014).

2.11 Peanut butter processing by SMEs in Tanzania

In Tanzania, about 27% of the gross domestic product and 23.4% of the total employment is contributed by 3 million small and medium enterprises (URT, 2012). Small Industries Development Organization (SIDO) has been conducting entrepreneurship programmes involving training on food processing, including peanut butter processing. The experience gained from SIDO has shown that the use of poor quality ingredients and poor manufacturing practices are among the sources of peanut butter spoilage, leading to a short shelf life of peanut butter and aflatoxin contamination (ITDG, 2002). Presence of defective nuts could be the source of aflatoxin contamination in groundnuts and its products (Ndungu *et al*., 2013). Unlike peanut grains, it is difficult to decide on the quality of peanut butter because peanut butter does not show any signs of mould, so no one can tell whether the grain used in processing was mouldy, insect damaged or contaminated (Samuel *et al*., 2016).

However, moulds are among the microorganisms which have been used in safety and quality assessment during peanut butter processing (Consumer report, 2009). The choice of raw material for peanut butter processing and hygienic practices during processing has effects on the quality and safety of the final product. Stages in peanut butter processing involve cleaning of groundnuts to remove unwanted materials; dry roasting of nuts for 10 to 30 minutes; cooling to stop cooking process; skinning and sorting for testa, and undesirable nuts removal. Furthermore, grinding of roasted nuts to smooth consistency paste and then additional ingredients (salt, sugar, vegetable oil and stabilizer) are added prior to butter filling in clean containers (ITDG, 2002).

2.12 Distribution and occurrence of aflatoxin

Poverty, drought, and adverse growing conditions in developing countries increase the risks of aflatoxicosis to the human population (Williams *et al*., 2004). The aflatoxigenic fungi are widely distributed in temperate and tropical areas. According to Tola and Kabede (2016), aflatoxigenic fungi can distribute in processing and storage facilities and in the distribution systems of the manufactured products. Peanut contamination can also occur on peanut marketing outlets, and therefore, a market survey would help to understand the market outlet contribution towards aflatoxin exposure (Mutegi *et al*., 2009).

2.13 Factors affecting mycotoxin contamination

The climatic conditions in African region favour the growth and proliferation of *Aspergillus flavus* and *parasiticus* (Williams, 2008). Several factors, including fungicides, physical factors like environmental, temperature, and humidity conditions as well as biological factors affect fungal growth which produces toxins in foods or feeds. Climatic conditions in developing countries, humidity and temperature, storage practices, transportation, and marketing contributes to the fungal growth and mycotoxin production in food crops (Kamika and Takoy, 2011). In a study carried out to assess the factors for aflatoxin contamination, it was reported that peanuts from agro-ecological zones with high humidity were more likely to be contaminated with aflatoxins than peanuts from agro-ecological zones with less humidity (Mutegi *et al*., 2009).

Pre-harvest crop contamination occurs in tropical and temperate conditions. In Eastern Africa, wet and humid conditions have been reported to allow fungal growth and toxin production (Kaaya *et al*., 2006; Mutegi *et al*., 2009). High temperature, high humidity, erratic rainfall during harvest and the timing of irrigation are the predisposing factors for fungal

infection on the field. Drought stress and temperature are major interrelated environmental factors that favour fungal infection in the soil (Bhatnagar *et al*., 2006; Payne, 1998). Under these conditions, toxigenic fungi auto-compete other plant and soil microflora, leading to their growth in peanut, corn, and cotton seeds. As kernels mature, low moisture and high temperatures favour the competitiveness of *aspergillus flavus* and *parasiticus*. Temperature alone or drought stress alone does not influence the increase of aflatoxin concentrations, but their dual effect on host and parasite interaction affecting both the fungus and host plant (Payne, 1998).

Specie morphologies also have effects on contamination such that S-strain of *aspergillus flavus* has been reported to strongly correlate with aflatoxin contamination in peanuts while no correlation of L-strain with contamination in peanuts was found (Mutegi *et al*., 2012). Favourable storage conditions for fungal growth results in post-harvest contamination (Williams, 2008). The risks of aflatoxin contamination in peanuts increase during post-harvest which is associated with poor handling practices along the chain (Kaaya *et al*., 2006). The risk of contamination during storage is also influenced by the fungal population, inadequate drying, environmental conditions, poor harvesting, and improper storage methods (Mutegi *et al*., 2009). Peanuts storage at 20 - 37^0C and high relative humidity (88 - 95%) under unseasonal rains result in intensive moulds growth and aflatoxins production (Omer *et al*., 2001).

2.14 Prevention and Control

Prevention and control of aflatoxin is very crucial towards protecting public health. Globally, various strategies for the control of mycotoxins have been considered, including the prevention of fungal growth before and after harvest, during storage, and processing.

2.14.1 Pre-harvest control

Prevention of mycotoxins on the field is very important to prevent mycotoxin development on crops which might proliferate into other stages.

(i) Resistant breeds/ improved cultivars

This is among the long-term strategy in control of mycotoxins in Africa. Various studies, for example, have been conducted to develop resistant peanut cultivar to *aspergillus flavus*. Azaizeh *et al*. (1989), tested seven peanut genotypes to determine *aspergillus flavus* growth on peanuts and determine drought stress treatment effects on the susceptibility of peanut

shells and kernels to fungal growth. The results showed variations in fungal growth among peanut genotypes caused by low moisture in the soil. Nevertheless, it was observed that shells colonization increased after harvest and kernels were more susceptible to *aspergillus flavus* and *aspergillus parasiticus*. In crop manipulation, Expressed Seguence Tags (ESTs) have been developed to identify aflatoxin resistant gene and control aflatoxin contamination (Guo *et al*., 2009).

Technology has been used in improving transgenic cultivar to control fungal contamination. Improved peanut varieties, for example, can reduce the incidence of fungal contamination (Mutegi *et al*., 2012). Also, Bacillus thuringiensis peanut gene has shown a significant reduction of aflatoxin levels than non-Bacillus thuringiensis peanut gene (Ozias-Akins *et al*., 2002). Equally, genetically modified crops that inhibit fungal growth to control infection have been developed (Wagacha and Muthoni, 2008). Furthermore, unlike unimproved local varieties which are associated with high aflatoxin levels, using improved seed varieties has been reported to reduce infection susceptibility by *Aspergillus* species and preventing crop damage (Mutegi *et al*., 2009). Most importantly, some organisations are still working on the development of resistant breed to control aflatoxins in Africa (Hell *et al*., 2005).

(ii) Biocontrol

Strategies towards the reduction or elimination of aflatoxins in crops have been explored including the development and use of bio competitive organisms in pre harvest control (Dorner *et al*., 1998). Organisms have been used as a means of biocontrol method to control aflatoxin contamination of crops in the field. Testing organisms such as yeast, atoxigenic, and bacterial strains for biological control of mycotoxins has been carried out (Yin *et al*., 2008). The use of biological atoxigenic fungi that compete with toxigenic fungi in inhibiting aflatoxin production has been reported to reduce the contamination levels (Turnel *et al*., 2005). The ability of atoxigenic fungi in controlling the toxigenic ones depends on the interaction between different effects of micro and macro climatic conditions. The introduction of atoxigenic *aspergillus flavus* and *aspergillus parasaticus* that strains into the developing crops resulted in a reduction of aflatoxin contamination of up to 99.9% in peanuts (Dornel *et al*., 1998).

(iii) Chemical control

Chemicals have been reported to effective in degrading structural and inactivating the aflatoxins, such as the use of oxidizing, chlorinating, alkali and hydrolytic agents

(Samarajeewa *et al*., 1990). However the combination of both chemical and physical treatments appears to be effective towards aflatoxins reduction (Samarajeewa *et al*., 1990).

During the production, the use of pesticides has a positive impact on minimizing fungal infection which consequently reduces mycotoxin contamination. While using fungicides during crop production has shown to be more effective towards fungal control (Turnel *et al*., 2005), their application is being limited due to food safety, economic, and environmental issues (Wagacha and Muthoni, 2008).

(iv) Proper harvesting

Early harvesting of crops reduces the risks of fungal contamination, though in Africa this practice tends to be difficult due to unpredictable weather conditions, the need for labour and cash, as well as the threat from animals leading to untimely harvesting of crops (Wagacha and Muthoni, 2008).

2.14.2 Post-harvest control

Various post-harvest strategies to control aflatoxin contamination have been implemented. Proper drying, physical separations like sorting, and the use of improved storage and insecticide during storage has been described as post-harvest approaches to reduce aflatoxin contamination. In a study conducted by Mohamed (2017), for instance, using improved storage, insecticide, and sorting has been proven to reduce aflatoxin contamination. Also, physical separation approach base on the removal of unwanted contaminants from the grain bulk which lower the contamination level (Wagacha and Muthoni, 2008). Proper drying of crops after harvest creates unfavourable conditions for fungal growth and toxin production. In a trial carried out in Guinea, drying and proper storage of groundnuts proved to reduce 60% of the aflatoxin levels (Turner *et al*., 2005). Moisture control on storage, during transportation and marketing to avoid contamination and the proper strategy towards the management of insect pests attack reduce the contamination of food crops (Wagacha and Muthoni, 2008)

2.14.3 During processing

Aflatoxins are stable metabolites that resist degradation during processing. In a dry form, their melting point is high ranging between 237^0C and 289^0C. Siwela *et al*. (2011) reported that during peanut butter processing, roasting temperature can change the toxin structure which reduces its concentration while blanching reduces 27% of the total toxins. However, their combination may result in 78% of total aflatoxins. Also, roasting during processing

helps aflatoxins reduction in peanut (Kaaya *et al*., 2006; Ndung'u *et al*., 2013). The control of processing methods and storage, therefore, can promote and contribute to the development of the peanut industry (Ding *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study site, design and sampling

The study was conducted in Arusha city located in the northern part of Tanzania. The site was chosen due to the availability of different brands of locally processed peanut butter. A simple random technique was used to collect the samples from retail market outlets, whereby a retail market survey was done to identify common processors of the peanut butter products. Out of which 10 common brands from different firms were identified and from each, 5 samples were collected from different retail selling points to make a total of 50 samples. The sample size was calculated by Power analysis using ANOVA to get the required number of samples suitable for the study.

3.2 Analysis of Aflatoxin in peanut butter

Aflatoxins were extracted from peanut butter samples by the method described by (Stroka *et al*., 2000), with external derivatization reported by (Tarter *et al*., 1984).

3.2.1 Extraction

The extraction procedure was the same for the vicam fluorometer and HPLC methods. Aflatoxins were extracted from peanut butter samples by the method described by (Stroka *et al*., 2000), alongside external derivatization as reported by (Tarter *et al*., 1984). A portion of 10 g of homogenized peanut butter sample was weighed and 1g of NaCl was added. Forty (40) ml of extraction solution (80% methanol and 20% water) as well as 20 ml of hexane were added and blended for two minutes and immediately filtered through Whatman filter No. 1. After that, 10 ml of filtrate was mixed with 70 ml of Phosphate Buffer Saline ready for clean-up procedure.

3.2.2 Clean-up procedures

A clean-up step is done to eliminate impurities prior detection. Immunoaffinity columns containing antibodies which bind the aflatoxins and impurities from the sample matrix were used. Immunoaffinity columns were activated with 10 ml of PBS and 80 ml of sample extract which was passed through the column fitted to a vacuum manifold. Then, 15 ml of water was used to wash the column and the column was dried further by slightly application of pressure. Eventually, aflatoxins from the column were eluted with 3 ml of elution solvent (99% methanol and 1% acetic acid) into a test tube with a maximum flow rate of 1ml/min.

3.2.3 Derivatization and Detection of aflatoxins by HPLC

(i) Derivatization

From the clean-up stage, the extraction eluate was evaporated to dryness with nitrogen gas, followed by derivatisation with 200 µl of n-hexane and 50 µl of trifluoroacetic acid that were incubated for 20 minutes, Then, the mixture was left for 10 minutes to a complete derivatization, and then dried with nitrogen. The residue was later re-dissolved with 200 µl of mobile phase (methanol: water: acetonitrile: acetic acid at a ratio of 23:57:20:0.1 v/v respectively).

(ii) Detection of aflatoxins by HPLC

This is the final stage in the determination of aflatoxins. After sample derivatization, the mixture was injected into HPLC for quantification.

3.2.4 Recovery and limit of detection for HPLC

A total of aflatoxin standard solution at different concentrations (33 and 100µg/kg) was added to the peanut butter sample in triplicate. The extraction of the spiked sample was done as described above and analysed by HPLC.

3.2.5 HPLC condition

HPLC fluorescence detection system was connected to Shimadzu auto-injector, a Shimadzu RF-20A fluorescence detector, column C18 (size 250×4.6mm), and autosampler SIL 20AHT. The methanol: acetonitrile: water (23:20:57 v/v) was used as a mobile phase with the flow rate of 0.3 ml/min and running time of 25 minutes. The oven temperature was set at 20°C. Fluorescence was set at wavelengths of 360 nm excitation and 440 nm emissions.

3.2.6 Detection of aflatoxins by Vicam fluorometer

Vicam series 4EX fluorimeter optical system with high-intensity pulsed xenon lamp, together with photodiode detectors, selected fluorescence excitation, and emission filters were used. After elution, 1 ml of elute was mixed with 1 ml of the aflatest developer in the cuvvetes ready for the aflatoxins detection by the calibrated Vicam fluorometer. Calibration was done using calibration standard vials, 2 ml methanol, 2 ml n-hexane, and 2 ml distilled water. The running time was 2 minutes per sample.

3.2.7 Recovery and limit of detection for vicam fluorometer

To test the sensitivity of the method, the total aflatoxin standard solution at different concentrations (5, 10, and 15ppb) were added to the blank peanut butter sample. The extraction of the spiked sample was done in triplicate as described above and analyzed in a calibrated fluorometer.

3.2.8 Data analysis

The data were coded in excel then imported to R software program for analysis. A significance difference in aflatoxin level between firms was carried out by Kruskal-Wallis rank sum test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Recovery and limit of detection for Aflatoxins detected by HPLC

The average percentage recoveries were 105.7%, 91.5%, 101.8%, and 94.5% for aflatoxins B1, G1, B2 and G2 respectively (See Table 1 below). These average recoveries are within the per cent required range of 70% to 110 % (Muscarella *et al*., 2009). The RSD for aflatoxin B1, G1, B2 and G2 were 6, 7, 10 and 18 respectively. The limits of detection for aflatoxins B1, G1, B2 and G2 were 0.219, 0.021, 0.219 and 0.211µg/kg respectively. Sample chromatograph for aflatoxins after HPLC quantification showing individual aflatoxins (B1, B2, G1 and G2) with their respective time of detection (as shown in the appendix 2).

Table 1. Recovery, limit of detection and relative standard deviation

AFLATOXIN	LOD µg/kg	% RECOVERY	RSD
B1	0.219	105.7	6
G1	0.021	91.5	7
B2	0.219	101.8	10
G2	0.211	94.5	18

4.2 Aflatoxins contamination of peanut butter analysed by HPLC

The results for total aflatoxin levels of peanut butter samples detected by HPLC are given in the appendix 1. After HPLC quantification, 48% of the samples were contaminated with total aflatoxins at a range of 1.00-1981.37 µg/kg. Unlike fluorometer which determines the total aflatoxins present in the sample, HPLC quantifies the individual aflatoxins B1, B2, G1 and G2 present in the sample. Aflatoxins B1 was detected in 40% of the samples with levels ranging from 25.98 to 300.39 µg/kg (mean, 54.95 µg/kg) that exceeded maximum aflatoxin B1 limit of 5 µg/kg in food. Aflatoxin B2 was detected in 32% of the samples with levels ranging from 1.01 to 34.20 µg/kg (mean 5.19 µg/k). Also, whereas G1 was detected in 28% of the samples with the levels ranging from 52.51 to 1,832.17 µg/kg (mean, 324.09 µg/kg), G2 was detected in 30% of samples with levels ranging from 1.00 to 27.03µg/kg (mean, 3.63 µg/kg). Forty-four per cent (44%) of the samples had total aflatoxin levels that exceed the maximum Tanzania limit for total aflatoxins which is 10 µg/kg (Kimanya *et al*., 2008).

The distribution of total aflatoxins as detected by HPLC is shown in Fig. 3 below. This figure show how aflatoxins detected in samples are widely distributed in various aflatoxin ranges.

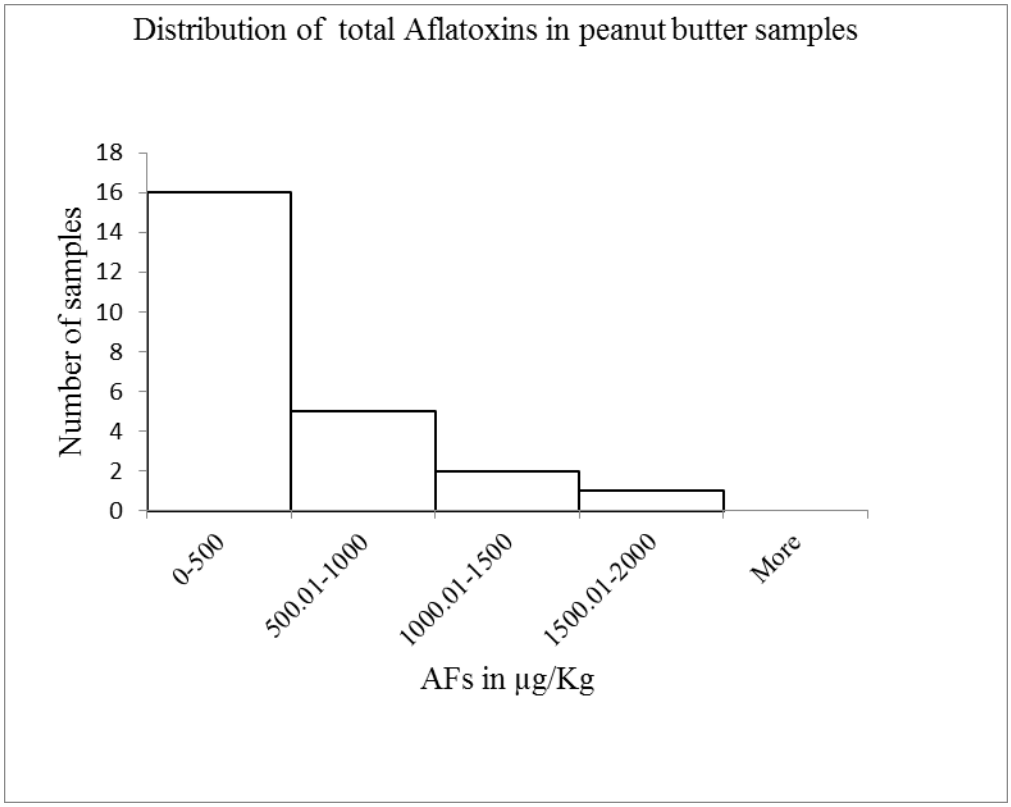


Figure 3: The overall distribution of aflatoxin in peanut butter samples as detected by HPLC.

4.3 Total aflatoxin levels between firms as detected HPLC

Detection by HPLC; the mean levels of total aflatoxins in samples from all firms ranged from 14.07 to 908.58 µg/kg (See Table 2 below). Numerically, the mean levels between firms differ, but statistically, the difference between the levels of total aflatoxins was found insignificant amongst 9 firms, that is p-value of 0.3607 ($p > 0.05$).

4.4 Recovery and limit of detection for Aflatoxins detected by Fluorimeter

The mean per cent recovery for total aflatoxins was 88.8% and the limit of detection was 2.4 µg/kg.

Table 2. Means of total aflatoxin content for each firm (HPLC)

Firm	Mean µg/kg ± SE
A	569.3 ± 543.3
C	312.2 ± 171.5
D	908.6 ± 393.9
E	124.1 ± 11.9
F	449.9 ± 423.9
G	68.1 ± 42.1
H	589.8 ± 322.8
I	26.9 ± 14.9
J	111.6 ± 84.6

$p>0.05$ (the difference between the levels of total aflatoxins was found statistically insignificant amongst 9 firms)

4.5 Aflatoxins contamination of peanut butter analysed by fluorometer

The results for total aflatoxin levels of peanut butter samples detected by fluorometer are given in the appendix 1. As detected by fluorometer, all peanut butter samples contaminated with total aflatoxins ranged from 5.6 to 720 µg/kg, while 92% of the total aflatoxins levels exceeded the maximum Tanzania limit for aflatoxins 10 µg/kg (Kimanya *et al*., 2008).

The distribution of total aflatoxins as detected by fluorometer is shown in Fig. 4 below. This figure shows how aflatoxins detected in samples are widely distributed in various aflatoxin ranges.

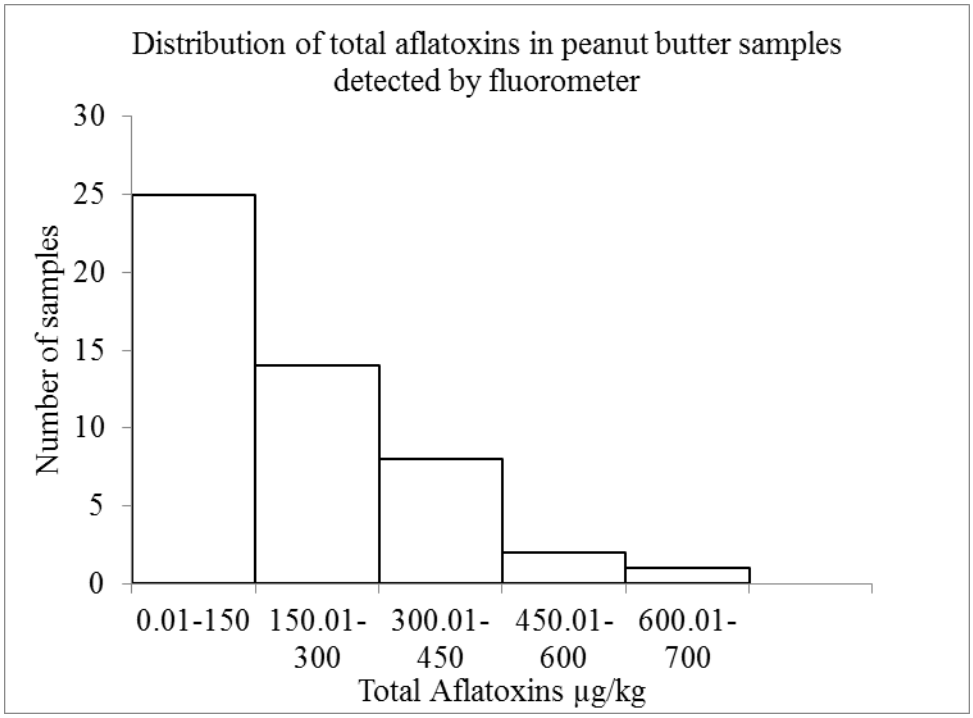


Figure 4: Overall distribution of aflatoxin in peanut butter samples (Fluorometer).

4.6 Total aflatoxin levels between firms as detected by Fluorometer

Detection by fluorometer; the mean total of aflatoxins levels from ten different firms ranged from 63.48 to 308 µg/kg (Table 3 below). Although there are numerical differences on the mean levels of aflatoxins between firms, statistically the analysis of variance indicated no significant difference in total aflatoxin level between them ($P > 0.05$).

4.7 Aflatoxins contamination of peanut butter analysed by fluorometer and HPLC

The results for total aflatoxin levels of peanut butter samples detected by HPLC and fluorometer are given in the appendix 1. A fluorometer gives total aflatoxins while HPLC detects individual aflatoxins present in the sample. The fluorometer has detected aflatoxins in all the samples, while HPLC has detected aflatoxins in 48% of the samples. This difference in aflatoxins quantification in samples might be due to sample pre-treatment prior analysis. Pre-treatment of the sample and method of extraction prior to determination has effects on aflatoxin recoveries in the sample (Asas *et al*., 2002). The longer time of sample extraction and external derivatisation process prior to HPLC quantification might be the reason for few aflatoxins detected samples as compared to the fluorometer.

Table 3. Means of total aflatoxin content for each firm (Fluorometer)

Firm	Mean ± SE
A	278±64.5
B	181.8±45.9
C	159.8±66.6
D	63.48±41.0
E	140.2±42.4
F	219.9±88.6
G	293.2±86.7
H	170.6±57.8
I	308±106.6
J	124.6±59.1

P value = 0.257, ($p>0.05$, there is statistical evidence that there is no significant difference in total aflatoxins between firms)

Globally, human populations are predisposed to the consumption of diets naturally contaminated with mycotoxins along the food chain. Studies have revealed an association between dietary consumption of aflatoxins and high incidence of liver cancer in Africa (Wagacha and Muthomi, 2008). Peanut is among the dietary staple foods that cause most of the mycotoxin poisoning problems in Africa. The ingestion of aflatoxin-contaminated food, for instance, has been reported to cause 250 000 hepatocellular carcinoma deaths annually. The contamination has also led to fatal aflatoxin outbreak which occurred recently in 2004, 2005, and 2006 in various countries in Africa (Lewis *et al*., 2005; Wagacha and Muthomi, 2008). In particular, fatal aflatoxin outbreak occurred in 2016 in Dodoma Region, Tanzania. Furthermore, the consumption of peanut butter contaminated with aflatoxin has been reported to be the risk factor for hepatocellular carcinoma in Sudan (Omer *et al*., 2001).

Worldwide, the set standard limits for aflatoxins B1 and total aflatoxins in food range between 1 and 20 µg/kg and from 1 to 35 µg/kg (FAO, 2004). World health organisation

(WHO) and Food and Agricultural Organisation (FAO) have adopted standard aflatoxin level of 15 µg/kg for raw peanuts and 10 µg/kg for processed nuts (CAC, 2001). Also, the Tanzania Bureau of Standard has set a maximum allowable limit of 5 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxins in food (TBS, 2004). However, aflatoxin standard setting does not guarantee the safety of food, particularly in developing countries where proper food inspection is rarely practised.

This study aimed at quantifying total aflatoxin levels in peanut butter sold in retail markets in Arusha City, Tanzania. The findings have revealed high levels of total aflatoxins contamination above the allowable maximum limit of aflatoxins in food (5 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxins) in Tanzania. The upper limit obtained using fluorometer method was 720 µg/kg, while for HPLC method, it was 1981.37 µg/kg. Other studies have reported the incidence of aflatoxin in peanut butter samples. In Haiti and Kenya, Filbert and Brown, (2012) reported aflatoxin contamination of all peanut butter samples with the levels of up to 799.8 ppb, which is almost the same level as that detected by fluorometer in this study. However, this is more than two times lower level as compared to that detected by HPLC. Equally, in Turkey, all samples were contaminated with total aflatoxins of up to 75.74 µg/kg (Yentur *et al*., 2006). Although the level exceeded the allowed total aflatoxin limit (10 µg/kg), the level is low compared to the upper aflatoxin levels obtained in this study. Levels of food contamination, therefore, vary depending on the area, agriculture practices, and climatic conditions (Williams *et al*., 2004).

Among the aflatoxins, Aflatoxin B1 is the most toxic one due to its lethal potent. It is classified as a group one carcinogenic to humans known for causing hepatocellular carcinoma due to its synergic action with hepatitis B or with fumonisins and ochratoxins (Kamika and Takoy, 2011). There is limited evidence of aflatoxin B2, G1, G2 and M1 to be carcinogenic (Ding *et al*, 2012). In this study, the levels of aflatoxin B1 detected in samples were very high (25.98 to 300.39 µg/kg), posing a health risk to the consumers. In other studies conducted in Sudan, peanut butter samples showed 100% positive aflatoxin contamination B1 (Elshafie *et al*., 2011). In Zimbabwe, high levels aflatoxin was detected in peanut butter samples with a predominance of aflatoxin B1 contamination of 6.3 to 528 ng/g that exceeded Zimbabwean aflatoxin B1 limit (5µg/kg) in foods (Mupunga *et al*., 2014).

Weather conditions in developing countries contribute towards a high level of aflatoxins since relative humidity, temperature, and moisture content are important factors for the growth of *Aspergillus flavus* and *Aspergillus parasiticus* (Wagacha and Muthomi, 2008).

Humidity and temperature have an influence on the level of aflatoxins contamination in products during crop growth and on storage (Yentur *et al*., 2006). Omer *et al*. (1998) reported the relationship between humid local storage conditions and high aflatoxin concentrations in peanut products. The high levels of contamination observed in this study may be attributed to improper processing and preservation practices as well as cross-contamination during processing (Ndung'u *et al*., 2013). Hell *et al*. (2000) further showed that storage and packaging materials for peanuts contribute to the higher level of aflatoxin.

The similarities in aflatoxin levels between firms reveal that the quality control required by the law is not practised among peanut butter processors. This study suggests that the quality of the peanuts/raw materials used, preparation practices, and processing might be similar among firms. The use of poor quality and mouldy peanuts during processing under poor hygienic conditions may be the cause for high aflatoxin levels in the final product (Elshafie *et al*., 2011). The processors of peanut butter products may not consider the quality of the raw material or perform the aflatoxins management practices to control contamination during processing (Filbert and Brown, 2012). The lack of quality control and protective measures in food chain systems and the negligence of good hygiene practices during food handling and preservations might be among the contributing factors (Wagacha and Muthomi, 2008).

During peanut butter processing Ndung'u *et al*. (2013) observed that stored roasted nuts before grinding pending customer order resulted in a high level of aflatoxins in peanut butter. Also, it was observed that during processing, cleaning of the grinder between peanut butter was not done and this had the influence towards increased levels aflatoxin due to cross contamination. On the other hand, the presence of a trained food technologist among one of the processors and the observation of hygiene during processing resulted in peanut butter free from aflatoxin.

Product contamination at all stages from farm to processing line affect the shelf life, quality, and safety of packed foods. Peanut butter processing involves, cleaning of peanuts, dry roasting, cooling, skinning and sorting, grinding and the addition of ingredients (salt, sugar, vegetable oil and stabilizer) prior to filling in containers (ITDG, 2002). Roasting, testa removal, and blanching can reduce aflatoxins to an acceptable level (Afolabi *et al*., 2015; Siwela *et al*., 2011).

Peanut butter is used widely in foods for children such as porridge, but the young are more vulnerable to aflatoxin effects (Siwela *et al*., 2011). Thus, considering the high aflatoxins

risks of exposure to humans, especially children, care should be taken during peanut butter preparation. Other studies reported that improved quality control practices by peanut products manufacture reduce the aflatoxins contamination (Oliveira *et al*., 2009). Laboratory segregated peanut butter samples had significantly reduced aflatoxin to an acceptable consumption level compared to those purchased from retail stores (Elshafie *et al*., 2011).

4.8 Correlation of total aflatoxins between HPLC and Vicam Fluorimeter

The correlation results between HPLC and fluorimeter are shown in Fig. 5. The correlation between these two methods showed the closeness of the relationship between the levels of aflatoxins detected by both methods. Although there are numerical differences in aflatoxin results obtained between the two methods, the analysis of the correlation between the analytic results from the same samples revealed that there was a moderate positive correlation ($r = 0.47$) between HPLC and Vicam fluorimeter methods. The variations in the aflatoxins levels in the samples detected by the two methods could be a reason for the moderate relationship observed between fluorometer and HPLC.

The scatter plots showed a positive relationship between the total aflatoxin levels detected by both methods. This indicates that the methods of analysis should take into consideration the economic factors, efficiency in time, and materials to be used (Gilbert and Anklam, 2002). Both methods were found to be effective due to their high recoveries of total aflatoxin contamination in peanut butter samples. Comparing between the two methods in terms of variations in time, the HPLC took longer per sample, although a sample automation injection was in use, the running time average for each sample was 25 minutes, while in fluorometer the running time per sample was 2 minutes.

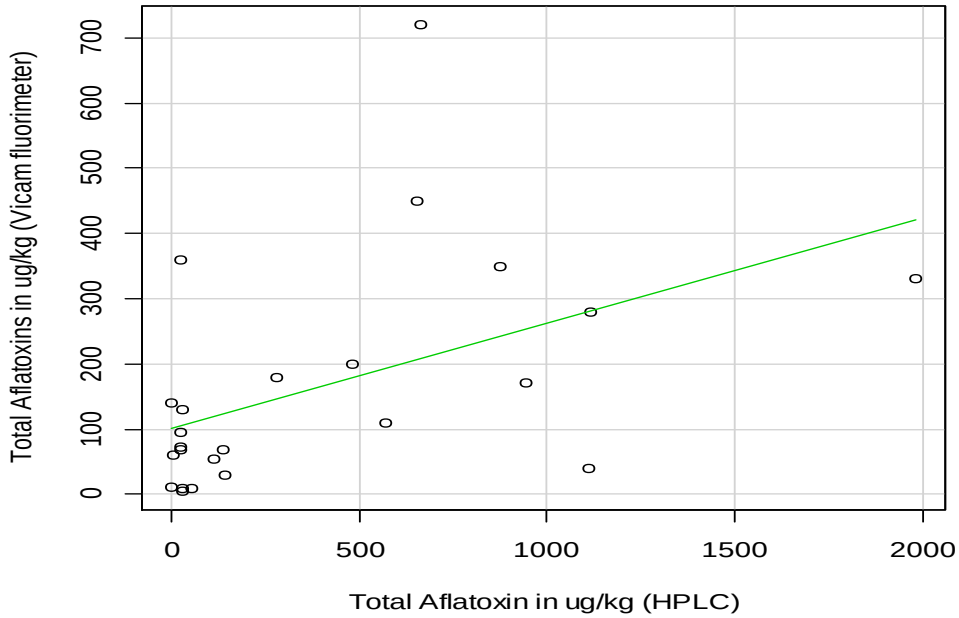


Figure 5: Correlation of total aflatoxins between Vicam fluorimeter and HPLC methods.

Although fluorometer is rapid, takes shorter time, and simple in analysis compared to HPLC, it should be ably capable of detecting the aflatoxins in samples. Due to the positive moderate relationship observed between the two methods, this study proposes that fluorometer could be a suitable and cost-effective pre-screening tool that can be used by food processors to timely identify aflatoxin contamination of their raw materials and products during the pre and post-processing stages.

4.9 Correlation of total aflatoxins with individual aflatoxin B1, B2, G1 and G2 detected by HPLC

Unlike HPLC which quantifies individual aflatoxins (B1, B2, G1 and G2) separately, fluorometer method gives a total aflatoxin content by the addition of a fluorescence developer that increases the fluorescence of total aflatoxins present in the samples. Correlation of total aflatoxins with individual aflatoxin B1, B2, G1 and G2 detected by HPLC are shown in Fig. 6, 7, 8, and 9. The correlation of total aflatoxins detected by fluorometer with individual aflatoxins detected by HPLC was moderate with aflatoxin G1 (r=0.48), weak with aflatoxin G2 (r=0.38), poor correlation with aflatoxin B1 (r=0.13) and B2 (r=1.8). The moderate correlation of total aflatoxins with aflatoxins G1 reflect that aflatoxin G1 was the

predominant toxin in the samples compared to other aflatoxins. It can also be seen in appendix 1 where the levels of aflatoxin G1 are very high compared to other aflatoxins.

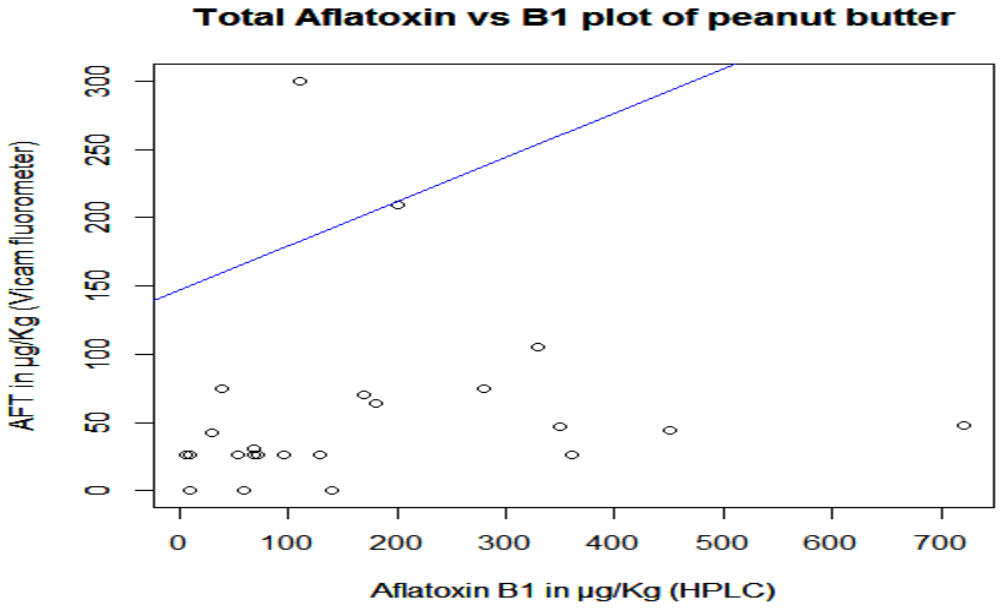
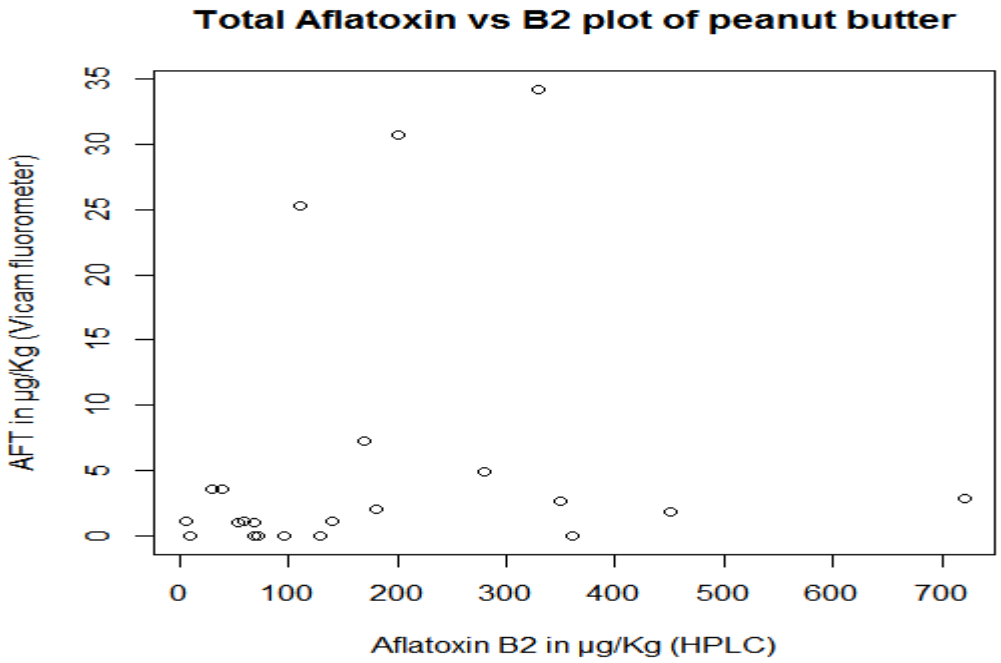


Figure 6: Correlation of total aflatoxins (fluorometer) and aflatoxin B1



(HPLC)

Figure 7: Correlation of total aflatoxins (fluorometer) and aflatoxin B2 (HPLC)

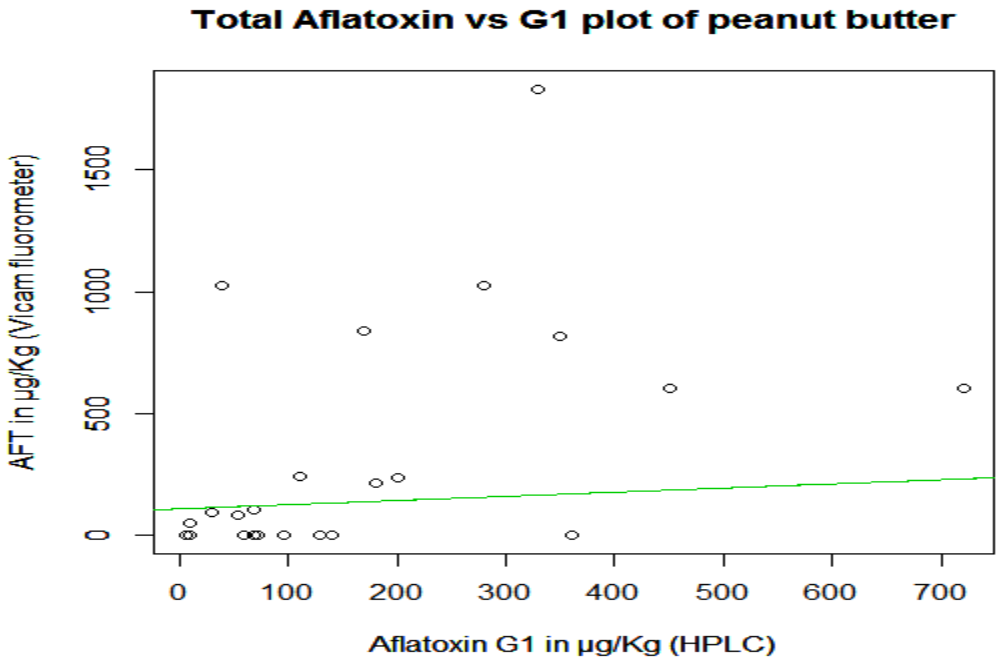


Figure 8: Correlation of total aflatoxins (fluorometer) and aflatoxin G1 (HPLC)

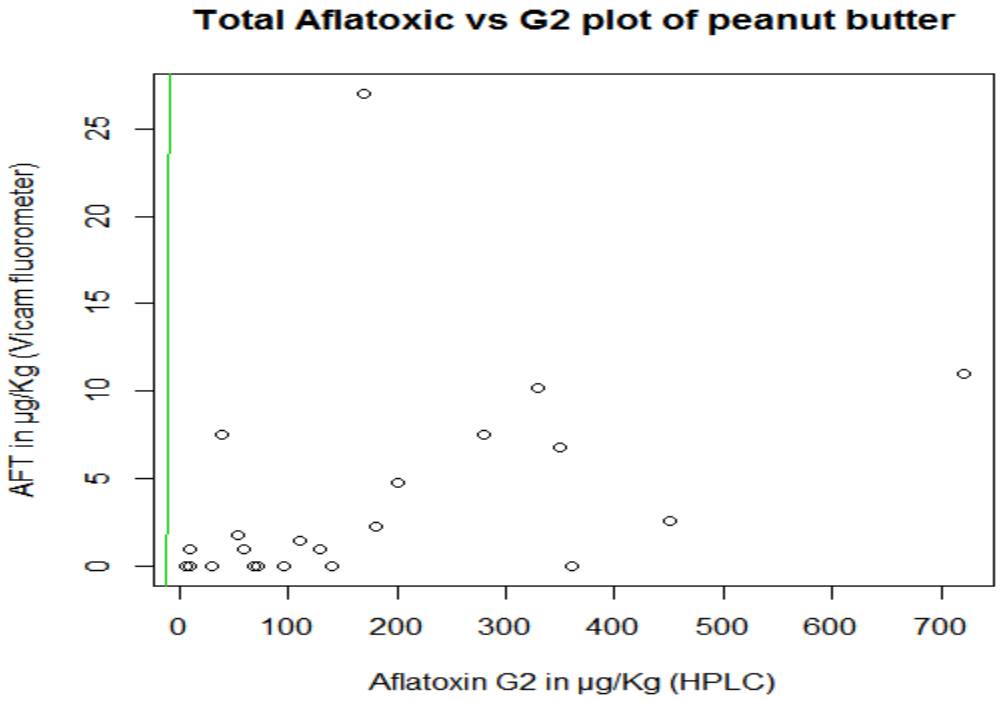


Figure 9: Correlation of total aflatoxins (fluorometer) and aflatoxin G2 (HPLC)

Generally, peanut contamination poses a health risk and has been identified as a major limitation to trade in Africa (Mutegi *et al*., 2009). An acute exposure of aflatoxins to humans leads to aflatoxicosis outbreak which significantly contributes to the burden of diseases in Africa (Gordon, 2003; Mehan *et al*., 1991). Continuous exposure to aflatoxins at low levels over a long period leads to chronic aflatoxicosis (Mehan *et al*., 1991). Chronic effects of aflatoxin exposure include a decrease in micronutrients absorption which, in turn, impairs food conversion and contributes to growth retardation (Jolly *et al*., 2007).

The presence of aflatoxins in peanut butter samples for human consumption calls for the aflatoxins management strategies, including quality control measures that will ensure the safety of products for human consumption. The persistence of aflatoxins under storage conditions as well as handling and processing of foods make impossible to eliminate the toxins in contaminated foodstuffs (Kabak, 2010; wang and Liu, 2007). This is mainly because aflatoxins are not affected by temperature and may stay active even at 160°C (Wagacha and Muthoni, 2008).

However, various strategies are being enforced to control aflatoxins during crop production and food preparation, including Good Agronomic Practices (GAP), timely crop harvesting, proper drying of crops for moisture control, physical separation like sorting, and the use of improved storage structures that prevents moisture inlets, insects, and rodents. Using biological control like atoxigenic fungi that compete with the toxigenic ones as well as resistant varieties which contain resistant genes towards fungal growth has been reported to minimize aflatoxins contamination (Bankole and Adebanjo, 2003). Processing flow monitoring from the farm, storage and processing, inspecting prior product market release, and using principles of Hazard Analysis and Critical Control Points during processing improve food safety (Calhoun, 2013).

High levels of aflatoxins contamination in peanut butter samples revealed in this study is alarming and might pose a health threat to the consumers. A long-term consumption of contaminated food is hazardous to human health. The aflatoxins' contamination of peanut butter products does not only pose harmful health effects to consumers but also leads to the significant loss of economy due to the loss of product`s export value (Yentur *et al*., 2006). Equally, the alarming level of peanut butter contamination calls for the regulatory bodies and food value chain actors to ensure that the control measures are in place so as to reduce exposure to and associated health problems. The continuous aflatoxin national surveillance and the creation of awareness intervention have to be advocated (WHO, 2006). It is also

important to sensitize stakeholders to take into consideration the mycotoxins prevention strategies along the food value chain.

Apart from the control and management of manufacturing practices, testing the products before and after processing prior to the market release is very important in assuring the safety and quality of the final product. The positive correlation between the two methods suggests that the fluorometer might be a suitable and cost-effective screening tool for aflatoxin levels in locally processed food products. This could help food processors to timely identify aflatoxin contamination of their raw materials before purchasing them, and hence, be able to produce the products free from aflatoxins.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results from both fluorometer and HPLC analytical methods revealed high aflatoxin levels in locally processed peanut butter products that are found in the market of Arusha City. The high contamination levels of aflatoxins pose a serious safety concern to the public as they are widely marketed and consumed in Arusha City. Lack of quality control and protective measures throughout the peanut butter production chain may be the contributing factors towards this contamination. This suggests that adequate care should be taken throughout the peanut production chain so as to produce peanut products which are safe for human consumption.

The study has also revealed positive moderate correlation between fluorometer and HPLC methods of analysing aflatoxins in peanut butter. This suggests that fluorometer method can be a suitable and reliable tool for the determination of aflatoxins in peanut butter that can be used at local processing unit. This is due to its shorter time of analysis and ability to detect the aflatoxins in the product at low operating costs that can be affordable to the local peanut butter processors as compared to HPLC method. It can help both small and medium peanut butter processors to examine the aflatoxin status of their raw materials prior to processing and of the final products.

Safety practices along the peanut value chain that is from the farm throughout production and storage will significantly minimize the aflatoxin contamination in food. The Tanzanian food safety and control organisations such as Tanzania Food and Drug Authority (TFDA) and Tanzania Bureau of Standards (TBS), alongside other stakeholders should be effectively involved towards the control and management of aflatoxins contamination in foods to protect the public health. Improving product safety and quality not only will protect the public health but will also improve the products market value, both locally and internationally. This will eventually help to gain export earnings that will contribute to public and national development.

5.2 Recommendations

Based on the findings of this study, I recommend the followings:

(i) The increase in local food processed products in Tanzania calls for more research to be conducted on the assessment of aflatoxin contamination in peanut butter products and the exposure assessment to determine the magnitude of aflatoxin exposure risks to humans.

(ii) Studying the effect of pre and post-harvest practices on aflatoxin contamination of peanuts in different environmental conditions in Tanzania.

(iii) The need for investigating the factors associated with the aflatoxin contamination during peanut butter processing by assessing the effect of processing practices towards aflatoxin contamination along the processing chain.

(iv) The adoption of aflatoxin prevention technologies throughout the peanut production chain and the generation of novel technologies that suit the agroecological conditions in Tanzania would help in the management of aflatoxin contamination.

(v) Implementation of aflatoxins control regulations by the food quality and safety control agencies in the country is highly recommended.

(vi) Creation of public awareness on the occurrence of aflatoxins in food, their effects to human and animal health, and the measures towards aflatoxins control. This can be done by conducting national campaigns on food quality and safety.

(vii) Education on mycotoxins control should be incorporated during peanut butter processing training programs such as training programs provided by SIDO to the SMEs so as to produce safe peanut butter products.

(viii) The food safety control authorities should continuously inspect the peanut butter processing areas so as to make sure that the products are always produced in a safe environment.

(ix) Diversification of food is important to reduce the exposure to aflatoxins, by using other food products such as fruit jams and margarine.

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APPENDICES

Appendix 1: Aflatoxin levels in peanut butter samples as detected by HPLC and fluorometer

	HPLC Results					Fluorometry results
Sample No	AFG2	AFG1	AFB2	AFB1	Total Aflatoxins µg/kg	µg/kg
1	2.5948	605.4668	1.8271	44.5460	654.4347	450
2	1.7995	80.8859	1.0146	26.4543	110.1543	54
3	11.0283	601.0676	2.9133	47.8555	662.8647	720
4	2.2430	212.8702	2.0145	63.5431	280.6708	180
5	6.8027	817.4363	2.7182	46.7578	873.7150	350
6	ND	ND	ND	ND	ND	270
7	1.0036	ND	1.1454	ND	2.1491	60
8	ND	ND	ND	25.9866	25.9866	73
9	27.0268	840.9547	7.2737	70.2160	945.4712	170
10	1.0021	ND	ND	ND	1.0021	10
11	ND	105.6540	1.0627	30.5266	137.2434	69
12	ND	ND	ND	26.0033	26.0033	360
13	ND	ND	1.1418	25.9812	27.1230	5.6
14	ND	ND	ND	ND	ND	240
15	ND	ND	ND	ND	ND	72
16	ND	ND	ND	ND	ND	110
17	ND	52.5123	ND	ND	52.5123	9.8
18	ND	ND	1.1526	ND	1.1526	140
19	ND	ND	ND	ND	ND	220
20	ND	ND	ND	25.9865	25.9865	69
21	ND	ND	ND	ND	ND	140
22	ND	ND	ND	ND	ND	130
23	ND	94.5763	3.6336	42.4658	140.6757	30
24	1.4827	243.1277	25.2277	300.3967	570.2348	110
25	ND	ND	ND	ND	ND	70
26	ND	ND	ND	ND	ND	420
27	4.8189	238.6686	30.7080	209.4800	483.6755	200
28	10.1918	1832.1738	34.2026	104.8026	1981.3707	330
29	7.5015	1026.4304	4.8864	74.9366	1113.7549	280
30	7.5030	1026.5746	3.5719	74.9962	1112.6457	40
31	1.0132	ND	ND	26.0017	27.0149	130
32	1.0029	ND	ND	25.9790	26.9819	9.5
33	ND	ND	ND	ND	ND	370
34	ND	ND	ND	ND	ND	91
35	ND	ND	ND	ND	ND	390
36	ND	ND	ND	ND	ND	180
37	ND	ND	ND	ND	ND	230
38	ND	ND	ND	ND	ND	280
39	ND	ND	ND	ND	ND	110
40	ND	ND	ND	ND	ND	600
41	ND	ND	ND	ND	ND	150
42	ND	ND	ND	ND	ND	180

43	ND	ND	ND	ND	ND	270
44	ND	ND	ND	ND	ND	190
45	ND	ND	ND	ND	ND	160
46	ND	ND	ND	ND	ND	54
47	ND	ND	ND	ND	ND	170
48	ND	ND	ND	ND	ND	85
49	ND	ND	ND	ND	ND	270
50	ND	ND	ND	25.9968	25.9968	96

Key: ND = Not Detected.

Appendix 2: Sample chromatograph for aflatoxins after HPLC quantification showing individual aflatoxins (B_1, B_2, G_1 and G_2) detection with their respective time of detection.