

**AFLATOXIN IN PEANUT BUTTER AND COMPLIANCE WITH THE
NATIONAL FOOD LAWS AMONG SMEs IN DAR ES SALAAM REGION**

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**A DISSERTATION SUBMITTED IN PARTIAL FULLFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD
QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF
AGRICULTURE. MOROGORO, TANZANIA.**

2019

ABSTRACT

The aim of this study was to determine the levels of aflatoxin in peanut butter and compliance with the national food laws among SMEs peanut butter manufacturers in Dar es Salaam region. Semi-structured questionnaire was used to assess awareness on aflatoxin contamination in peanut butter, and observation checklist was used to assess compliance practices with national food laws among SMEs peanut butter manufacturers. Samples of peanut butter were collected randomly from 30 peanut butter SMEs selected from the list provided by Small Industries Development Organization (SIDO). Samples were analyzed by High Performance Liquid Chromatography (HPLC) with fluorescence detection. The total amount of aflatoxins detected in peanut butter samples ranged from 0.075 to 317 $\mu\text{g}/\text{kg}$ of which 33.3% of the samples for AFB1 (5.1 to 277.7 $\mu\text{g}/\text{kg}$) and 26.6% of the samples for total aflatoxin (15.5 to 317 $\mu\text{g}/\text{kg}$) exceeded the TBS regulatory limit 5 $\mu\text{g}/\text{kg}$ and 15 $\mu\text{g}/\text{kg}$ respectively as established by TZS 844:2014. The results revealed that all peanut butter SMEs had knowledge on susceptibility of peanut and peanut butter to aflatoxin while only 3.3% of peanut butter SME was not aware on favorable conditions for aflatoxin in peanuts. These peanut butter SMEs did not register 80% of the premises and 83.3% of the products, and most 90% of the products did not have TBS marks. Also 56.7% of peanut butter SMEs did not have business license. The high level of aflatoxin found in peanut butter poses significant threats to the health of consumers. Therefore, food regulatory authorities should encourage SMEs peanut butter manufacturers to use clean raw material and practice required Quality Assurance (QA), and should monitor the levels of aflatoxin in peanut butter and ensure all SMEs peanut butter manufacturers are registered and comply with the national food laws.

DECLARATION

I, **LULU KIWIA** 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.

LULU KIWIA
(MSc. Student)

Date

The above declaration is confirmed

Dr. B. M. Kilima
(Supervisor)

Date

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for giving me strength, wisdom and good health which enabled me to pursue my studies and finish the research work.

I am greatly thankful to my supervisor; Dr. B. M. Kilima whom I am obliged to thank her for taking time to reading through the draft reports, and all the constructive criticisms and suggestions which have helped me to the successful completion of this dissertation. Special thanks goes to Dr. R. J. Mongi for his guidance in data analysis and presentation of my study.

I would also like to express my gratitude and appreciation to my employer as well as my sponsor Government Chemist Laboratory Authority for financing my study. I would specifically like to thank Mr. Emanuel Bakashaya and Mr. Wiseborn Makaka of TBS food Laboratory for their guidance and assistance during laboratory analysis.

Special thanks are extended to my lovely husband Mr. George P. Shoo for his encouragement and constant love, without forgetting my aunt Hawa H. Dachi, Eric H. Kiwia (brother) and Rose H. Kiwia (sister) for their moral support which motivated me to continue pursuing my study.

I applaud support and cooperation from my classmate whom we worked as a team during our studies. I acknowledge the academic staffs from Department of Food Technology, Nutrition and Consumer Sciences at SUA for their support. It would not be possible to acknowledge all individuals who supported me during my study. Lastly I extend my sincere appreciation to Mr. Jonathan Mbailuka from SIDO.

DEDICATION

This dissertation is dedicated to my lovely children, Milca and Ethan, my late parents Hayness W. Kiwia (father), Asha H. Dachi (mother), and my fellow GCLA workers.

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ABBREVIATION AND ACRONYMS

AFB1	Aflatoxin B ₁
AFB2	Aflatoxin B ₂
AFG1	Aflatoxin G ₁
AFG2	Aflatoxin G ₂
FAO	Food and Agriculture Organization
g	gramme
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
ITDG	Intermediate Technology Development Group
ml	millilitre
°C	Degree Centigrade
PACA	Partnership for Aflatoxin Control in Africa
QA	Quality Assurance
SIDO	Small Industries Development Organization
SME	Small and Medium Enterprise
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards

TFDA	Tanzania Food and Drugs Authority
TZS	Tanzania Standard
µg/kg	Microgram per kilogram
UNIDO	United Nations Industrial Development Organization
URT	United Republic of Tanzania
USA	United States of America
UV	Ultra Violet
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Peanut or groundnut (*Arachis hypogaea*) is a plant which belongs to the family (*fabaceae*) of bean/legume (Arya *et al.*, 2016). It is considered nutritious, as it contains proteins, oils, fatty acids, carbohydrates, and minerals (Settaluri *et al.*, 2012). In Tanzania, peanuts are mainly grown in Dodoma, Singida, Tabora, Mtwara, Shinyanga and Mwanza regions by smallholder farmers (Bucheyeki *et al.*, 2010). Peanuts are widely used for production of varieties of products including peanut butter, oil, confections and roasted peanuts (Arya *et al.*, 2016).

Peanut butter is made by grinding dry roasted peanuts into a paste, it contains a minimum of 90% peanuts. Sweeteners and salt can be added to enhance flavor while small amounts of stabilizers are used to prevent oil separation (Akhtar *et al.*, 2014). However, peanuts are liable to colonization by fungal molds during handling, storage and transportation which expose peanut butter to the risk of contamination with Aflatoxin (Villa and Markaki, 2009; Mutegi *et al.*, 2012).

Aflatoxins are a group of naturally occurring mycotoxins that are produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus* that typically infect food crops such as peanuts used in manufacturing of food products including peanut butter (Patel *et al.*, 2015). According to Mmongoyo *et al.* (2017) fungi produce four main types of aflatoxins which include aflatoxin B₁ [AFB₁], B₂ [AFB₂], G₁ [AFG₁], and G₂ [AFG₂]. The International Agency for Research on Cancer (IARC), (2002) has classified

“naturally occurring mixtures of aflatoxins” (B₁, B₂, G₁ and G₂) as a class 1 carcinogen known to cause cancer in humans.

Most governments including Tanzania have established food laws and regulations to protect consumers from harmful effects of aflatoxins (FAO, 2004). Tanzania Food and Drugs Authority (TFDA) and Tanzania Bureau of Standards (TBS), require manufacturers of food products to comply with national food laws in order to process food with safe and of high quality. Food Manufacturers are required to have business license, premises and products registration, and product TBS mark. Government authorities are also required to create awareness among producers and consumers on the aflatoxin contamination in food products to include peanut butter products.

There is inadequate information on aflatoxin contamination in peanut butter in Tanzania, as well as compliance to national food laws among peanut butter SMEs. In Tanzania, most of the documented studies were on aflatoxin contamination in peanuts and cereals such as maize (Kimanya *et al.*, 2014; Magembe *et al.*, 2016). Therefore, this study aimed at assessing levels of aflatoxin contamination in peanut butter and compliance with the established national food laws.

1.2 Problem Statement and Justification

Contamination of peanuts with aflatoxins is a worldwide problem that affects both food safety and agricultural economics (Dorner, 2008). In Africa, environmental conditions especially high humidity and temperatures favor fungal proliferation in food of which may result into aflatoxin contamination (Wagacha and Muthomi, 2008). Dar es Salaam is among the Tanzanian region which experiences high temperature therefore it is prone to aflatoxin contamination.

Studies reveals that peanut butter and blended product are notably contaminated with high level of aflatoxins (Mupunga *et al.*, 2014; Shirima *et al.*, 2013). This is due to the fact that peanuts and its products such as peanut butter contain proteins, oils, fatty acids, carbohydrates, and minerals which are favorable medium for fungal growth and aflatoxin contamination (Settaluri *et al.*, 2012; Barberis *et al.*, 2012). Aflatoxins are characterized by their high toxicity which causes mutagenic and carcinogenic effects of which liver is considered as the main affected organ (de Oliveira and Corassin, 2014).

There is an increased use of processed food products including peanut butter from Small and Medium Enterprises (SMEs) in Tanzania (UNIDO, 1999). Foodstuffs including peanut butter products may contain high concentrations of aflatoxins which may result into haemorrhage, edema, digestion problem, liver damage and liver cancer (Njoroge *et al.*, 2016; Sarma *et al.*, 2017). However, there are scarce scientific studies on the levels of aflatoxin contamination in peanut butter particularly in region of Dar es Salaam.

Most of SMEs in Tanzania are not registered and they operate informally (Mohamed and Mnguu, 2014; Yahya and Mutarubukwa, 2015). This means that they produce without any form of regulatory control. Peanut butter manufactures are among the SMEs in Dar es Salaam region in which this study aimed to establish their compliance with the existing national food laws. Therefore, this study focused on determination of aflatoxins in peanut butter and compliance among SMEs with established national food laws in Dar es Salaam region.

1.3 Objectives of the Study

1.3.1 General objective

The general objective was to assess aflatoxins contamination in peanut butter and compliance with the national food laws among SMEs peanut butter manufacturers in Dar es Salaam region.

1.3.2 Specific objectives

- i. To determine the level of aflatoxins contamination of peanut butter processed by SMEs in Dar es Salaam region.
- ii. To assess peanut butter SMEs awareness on aflatoxin contamination in peanut butter.
- iii. To assess compliance of SMEs peanut butter manufacturers with established national food laws.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Small and Medium Enterprises (SMEs)

Food processing activities have grown in Tanzania in importance to meet new consumer demand, whereas most of the food manufacturing industries are small and medium enterprises (UNIDO, 1999). SMEs sector in Tanzania has been recognized as a base for the industrial development, employment creation, income generation, and poverty alleviation (URT, 2012). There is no unique, universally accepted definition for small and medium enterprises (Berisha and Pula, 2015; Gbandi and Amisah, 2014). Different countries use various measures of size depending on their level of development. In Tanzania, Small and Medium Enterprises (SMEs) are classified as micro enterprises (1 to 4 employees), small enterprises (5 to 49 employees), medium enterprises (50 to 99 employees) and large enterprises is above 100 employees (URT, 2012).

2.2 Peanuts

Peanuts are important crops in Tanzania, providing both nutrition and income (Kuhumba *et al.*, 2018). In Tanzania, peanuts are mainly grown in Dodoma, Tabora, Mtwara, Mwanza, Shinyanga and Sigida, regions by smallholder farmers (Bucheyeki *et al.*, 2010).

Peanuts (*Arachis hypogaea*) are major source of protein and edible oil, it contains 26% protein, 49% oil, 16% carbohydrate and 2.3% ash (Shem-Tov *et al.*, 2012). Peanut is an excellent source of vitamin E and also contains good amount of folate (Arya *et al.*, 2015). It has a source of calcium, magnesium, potassium, zinc, iron, phosphorus, thiamine, riboflavin, and niacin (Surendranatha *et al.*, 2011). It has notably been the source of elimination of malnutrition amongst the population in many African countries (Guimon and Guimon, 2012). Peanuts are an excellent source of compounds like resveratrol,

phenolic acids, flavonoids and phytosterols that block the absorption of cholesterol from diet (Arya *et al.*, 2015). Peanuts are consumed whole, boiled, roasted and as a variety of products including peanut butter, peanut flour, peanut oil, and other forms as ingredients (Chang *et al.*, 2013).

2.2.1 Peanut butter

In Tanzania, peanut butter is traditionally used for cooking purposes in many homes in rural areas. It is used in preparation of side dishes and is also cooked with vegetables, pearled sorghum and maize. Commercially produced peanut butter is used as a spread on bread especially in the urban homes (Cheng, 2016).

2.2.2 Peanut processing into peanut butter

Peanut butter is made by grinding dry roasted peanuts into a paste, it contains a minimum of 90% peanuts. Sweeteners and salt can be added to enhance flavor while small amounts of stabilizers are used to prevent oil separation (Akhtar *et al.*, 2014).

Stages in peanut butter processing involve cleaning of peanuts to remove unwanted materials such as dust, twigs and damaged peanuts; this is achieved by winnowing and blowing away the light particles. Broken and infested nuts are removed by hand of which sorting out physically damaged and infected grains from produce can result in 40 to 80% reduction in aflatoxin levels (Park, 2002). Then roasting of peanuts for 10 to 30 minutes with adequate heat to obtain uniform light brown to brown peanuts; cooling roasted peanuts; then skinning and sorting of peanuts where peanuts are rubbed and winnowed to separate the skins from the kernels, burnt and undesirable peanuts are removed. Moreover grinding of roasted peanuts to obtain a finely ground smooth paste, and then additional ingredients such as salt, sugar, vegetable oil (to improve flavor) and stabilizer (to prevent oil separation) may be added, where the paste with added ingredients is then heated to

about 80°C to ensure that the stabilizer melts and is well blended into paste prior to butter filling in clean containers (ITDG, 2002).

2.3 Aflatoxin

Aflatoxins are secondary metabolites of fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Wild and Gong, 2010). Aflatoxins commonly contaminate a variety of staple foods including peanuts, tree nuts, maize, milk and dried fruits and cause an array of acute and chronic human health disorders. Aflatoxin B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) are the four major types (Wu *et al.*, 2013). Aflatoxins designated by B1 and B2 show strong blue fluorescence under UV light, whereas the G1 and G2 forms show greenish yellow fluorescence (Wogan, 1966). AFB1 occurs mostly in foods including peanut butter which is highly toxic, carcinogenic, mutagenic and teratogenic compounds (Bakirdere *et al.*, 2012).

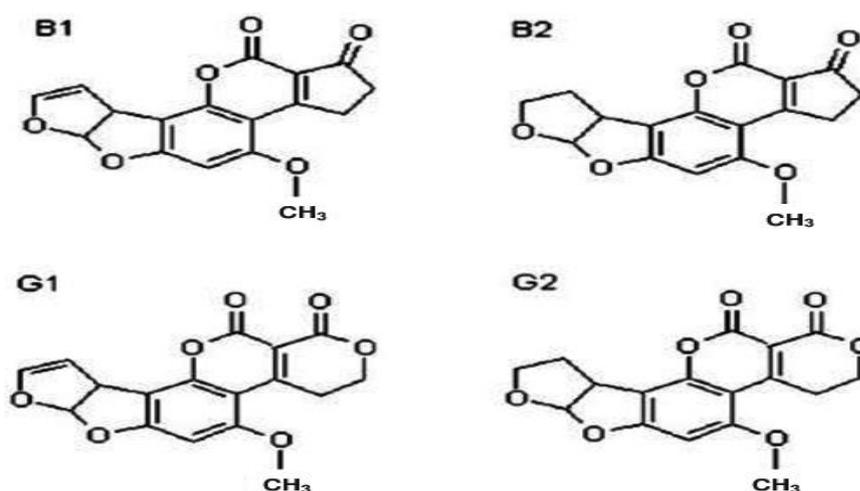


Figure 1: Chemical structure of aflatoxins B1, B2, G1 and G2

Source: (Martins *et al.*, 2013)

One of the major problems in peanut production worldwide is aflatoxins contamination, which is of great concern as these toxins have toxicological effects (Torres *et al.*, 2014). This is due to the fact that peanuts and its products such as peanut butter contain proteins, oils, fatty acids, carbohydrates, vitamins and minerals which are favorable medium for fungal growth and aflatoxin contamination (Settaluri *et al.*, 2012; Barberis *et al.*, 2012). The aflatoxins occur mostly in tropical regions with high humidity and temperature and they accumulate post-harvest when food commodities are stored under conditions that promote fungal growth (Wild and Gong, 2010).

Practices such as poor storage and handling within the peanut butter industry can contribute to further aflatoxin contamination of peanut butter (Ndung'u *et al.*, 2013). Studies have noted that storing roasted and or crushed peanuts in plastic bags at ambient temperature without grind it immediately had contributed to aflatoxin contamination in peanut butter (Ndung'u *et al.*, 2013; Elzupir *et al.*, 2011). Also, poor knowledge of the aflatoxin contamination may cause poor quality peanuts and peanut butter (James *et al.*, 2007). The optimal conditions for fungal growth and aflatoxin contamination are frequent in peanut crop fields as well as in storehouses (Abbas *et al.*, 2013). The rate and level of contamination depends on storage period, high relative humidity, high temperature, moisture content, harvesting and transportation (Kana *et al.*, 2013; Villers, 2014; Patel *et al.*, 2015; Hell *et al.*, 2010). The optimal condition for its growth was reported to be at the temperature of 30 – 33°C, relative humidity of 83 – 85%, water activity at 0.83 – 0.97 and moisture content >14% (Norlia *et al.*, 2018).

2.3.1 Aflatoxin contamination levels

Aflatoxin contamination levels in cereals and foods vary between different countries, with limits ranging from 0 to 35.0 µg/kg (Hoeltz *et al.*, 2012). The Codex Alimentarius

Commission, Joint FAO/WHO Food Standards Program set a limit of 15 µg/kg for total aflatoxin in foods including peanut butter (Codex, 2001).

According to Tanzania Standard (TZS 844:2014) limit for aflatoxin in peanut butter are 15µg/kg for total aflatoxin (B1, B2, G1, G2) and 5 µg/kg for aflatoxin B1 (AFB1). William *et al.*, (2004) reported that much of Sub-Saharan Africa countries are at risk of unsafe levels of aflatoxin exposure that can negatively affect human health, food security and economic trade. Mutegi *et al.* (2010) evidenced that high aflatoxin level up to 22 µg/kg in peanut products such as roasted peanuts and peanut butter have been reported in Nairobi, Kenya whereas the limit for aflatoxin are 10 µg/kg for total aflatoxin and 5 µg/kg for AFB1.

A study conducted in Tanzania showed that aflatoxin (AFB1) present in 20% of peanut samples from Manyara and Mtwara and in 8% of samples from Shinyanga were above the maximum tolerable limit of 5 µg/kg. The mean contamination levels in the samples that exceeded the legal limit were 20 µg/kg for Manyara and Shinyanga and 18 µg/kg for Mtwara (PACA, 2016). In Tanzania, aflatoxin susceptible foods such as peanut butter are widely consumed therefore acts as main sources of aflatoxin exposure (PACA, 2016). However, there is insufficient scientifically established data on the magnitude of the aflatoxin contamination in peanut butter in Tanzania.

2.3.2 Health effects of aflatoxin contamination

The main route of exposure to aflatoxin is through the direct consumption of contaminated food (Gong *et al.*, 2016). Aflatoxin species are highly dangerous compounds for human being; they can cause various adverse health effects depending on the level of exposure. High level exposure of aflatoxin that occurs over a relatively short

period of time is recognized as causing acute effects. Chronic effect is due to low dose aflatoxin exposure over a long period of time which is more prevalent than acute effects (Gong *et al.*, 2016). Chronic aflatoxin exposure has been associated with effects on immune function, hepatocellular carcinoma (liver cancer) and child growth impairment, as well as other effects such as hepatomegaly (Turner *et al.*, 2003; Gong *et al.*, 2012; Gong *et al.*, 2016). Wagacha and Muthomi, (2008) reported that in 2004, more than seven districts in Kenya experienced aflatoxin-poisoning outbreak with 317 case-patients and 125 deaths.

2.4 Food Law

FAO (2005) defines food laws as regulation of food control, food safety and food trade at national level and also focus on laws and regulations that refer to food in general or to specific kinds of food. Tanzania has a number of laws and regulations governing food processing and production, and quality control for both large-scale manufacturers and SMEs. The main relevant national food laws, regulations and guideline are under Tanzania Food and Drugs Authority (TFDA) and Tanzania Bureau of Standards (TBS) which establishes compliance requirements to minimize and/or prevent contamination of food and foods products to include aflatoxin contamination.

2.4.1 Tanzania Food and Drugs Authority

The Tanzania Food, Drugs and Cosmetics Act, 2003 requires food manufacturers to register their products and obtain permits for production, distribution and selling of their products. TFDA is mandated to analyze the food products and provide food registration certificate for food products intended to be registered in Tanzania in order to ensure that they comply with the safety and quality requirements. After registration, manufacturers of food products are supposed to obtain permits after fulfilling specific requirement for

manufacturing of food to include adherence to Good Manufacturing Practices (GMP). Also, food manufacturers are supposed to obtain certificate of registration of the premises and permits to carry out such food business. The general food premise requirements according to TFDA regulation are premises design, equipment, utensils and food contact surface, personnel, storage facilities, sanitation and hygiene. However, TFDA no longer exists, its responsibilities related to food were shifted to TBS.

2.4.2 Tanzania Bureau of Standards

TBS is mandated to establish standards under the Tanzania Standards Act, 2009 and perform its duties with intention of ensuring health safety and general welfare of the people of Tanzania. The Bureau is charged to ensure quality control of the commodities including foods by promoting standardization in industries and trade. The body set food standards and oversees observance of the standards. TBS marks are issued to the food products after food manufacturers fulfilling required standards to their products.

2.4.3 Compliance with food laws

Food manufacturers includes SMEs peanut butter manufacturers are required to comply with the requirements set by the food regulatory authorities (TFDA/TBS) in order to ensure that food products are safe for human consumption and meet prescribed quality parameters. In Tanzania, manufacturers of food products are required to meet compliance requirements which include hygiene of the food processing place, storage of product and raw material, possession of business license certificate, possession of product and premise registration certificates, and attainment of product TBS mark.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Dar es Salaam region. The choice of this area was based on the fact that Dar es Salaam is the industrial and commercial capital of Tanzania. The Region consist of five different municipalities namely Temeke, Ilala, Kinondoni, Ubungo and Kigamboni.

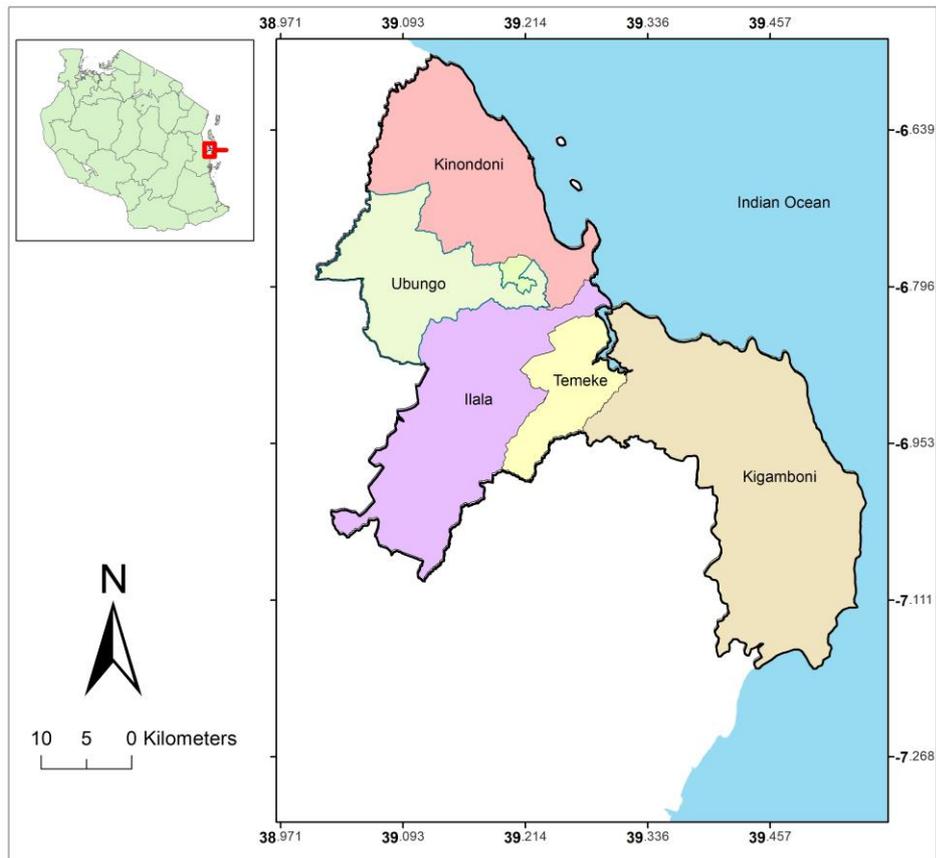


Figure 2: Map of Dar es Salaam region showing Municipalities where samples were collected.

3.2 Study Design

This is a cross sectional study design which involved survey and laboratory work. SMEs peanut butter manufacturers were selected randomly from the registered list provided by Small Industries Development Organization (SIDO). Semi-structured questionnaire was used to assess awareness of aflatoxin contamination in peanut butter (Appendix 1) and checklist was used to assess compliance practices with national food laws among peanut butter SMEs (Appendix 2).

3.3 Peanut Butter Sample Collection

A total of 30 peanut butter samples produced by SMEs in Dar es Salaam region were collected randomly and analyzed in duplicate. One sample of peanut butter was collected randomly from each of identified SMEs peanut butter manufacturer. Collection of sample was done from December 2018 to February 2019. The samples were coded and transported to the food laboratory of the Tanzania Bureau of Standards in Dar es Salaam and kept in dark, dry and cool place in the food laboratory until analyzed.

3.4 Determination of Aflatoxin Levels

Reagents used included: HPLC grade water, acetonitrile and methanol (HPLC grade), (all from Fisher Chemical, Bishop Meadow Road, Loughborough, Leicestershire), immunoaffinity columns and aflatoxins standards (AFB₁, AFB₂, AFG₁ and AFG₂) (AflaTest from Romer Labs GmbH, Technopark 13 430 Tulin, Austria).

The determination of aflatoxins (B₁, G₁, B₂, G₂) was carried out by the Standard Operating Procedure (SOP) no. FCL/SOP-TM/13-02 which followed Romer Labs procedures for the purification of aflatoxins in conjunction with High Performance Liquid Chromatography (HPLC). 25g of mixed peanut butter sample was weighed using the

calibrated analytical balance into a 250ml Erlenmeyer flask. Using a measuring cylinder, 100ml of methanol: water (70:30 v/v) as extraction solvent was added to the 250ml Erlenmeyer flask containing the sample. The flask was covered with aluminium foil and placed on the gyratory shaker (Stuart® Orbital Shaker SSL1, Cole-Parmer LLC, USA) at 250rpm/30 min, then using a filter paper Whatman No. 1, the extract was filtered into a 250 ml Erlenmeyer flask. The extracted sample (4 ml) was transferred to 15ml centrifuge tube, then diluted by adding 8ml of distilled water. The mixture was vortexed (Talboys® Hvy Dty Vortex, Troemner LLC, USA) for 1 minute to get a homogeneous mixture, followed with clean up stage where diluted extract was loaded and allowed to pass through Solid Phase Extraction (SPE) immunoaffinity columns and the sample loaded columns were rinsed twice with 10 ml of HPLC grade water. In elution stage, the adsorbed aflatoxins were eluted with 1 ml of HPLC grade methanol and the eluents were collected in vials. Finally, pressure was slightly applied on top of the column to remove any remaining liquid, then 0.3ml of the eluate was mixed with 0.6ml of water and 0.1ml of acetonitrile and the mixture was vortexed for 30 seconds (ISO 16050: 2003).

HPLC system

After extraction, dilution, clean up and elution and post-column derivatization, the extracts were analyzed using HPLC with fluorescence detector (Model Agilent ChemStation technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). The mobile phase contained water: methanol: acetonitrile (60:30:10, v/v). The separation of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) was performed on the C₁₈ column at a temperature of 30°C at a flow rate of 1.2 ml/min. The injection volume was 50 µL for both standard solution and sample extracts. After separation, AFG₁ and AFB₁ were derivatized to allow their detection with fluorescence detector at an emission wavelength of 465 nm and an excitation wavelength of 360 nm.

The sample concentration was calculated as follows:

Concentration of the sample ($\mu\text{g}/\text{kg}$) =

$$\frac{3.33\text{mls} \times 100\text{mls} \times 1\text{ml}}{4\text{mls} \times \text{weight of the sample taken (g)}} \dots\dots\dots (1)$$

3.5 Statistical Data Analysis

Data from the laboratory analysis were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistical analysis was done to compute frequency distribution and proportions of peanut butter SMEs awareness on aflatoxin contamination in peanut butter as well as compliance to national food laws. Analysis of Variance (ANOVA) was used to determine significant difference ($p < 0.05$) in the aflatoxin levels between peanut butter SMEs, and regulatory limits. Means were separated by Duncan Multiple Range Test (DMRT). Whereas; results were presented in tabular and graphical forms as frequency, percent and mean \pm standard deviations.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter presents findings and discusses results of this study according to the specific objectives which covers the status of levels of Aflatoxin in peanut butter from SMEs peanut butter manufacturer, awareness on aflatoxin contamination in peanut butter and compliance of peanut butter SMEs with established national food laws.

4.1 Level of Aflatoxins Contamination of Peanut Butter Processed by SMEs

4.1.1 Basic information of the SMEs peanut butter manufacturers

Thirty three percent of the peanut butter SMEs were from Ilala municipality while about 23% were from Ubungo, (17%) Kinondoni, (17%) Temeke and (10.0%) Kigamboni municipalities (Figure 3a). About 70% of peanut butter SMEs had number of employees up to 4 while 30% had more than 4 employees (Figure 3b). This observation is in line with the findings from National Baseline Survey Report, (2012) which shows that most of manufacturing enterprises including food manufacturing in Tanzania are micro manufacturing enterprises with up to 4 employees.

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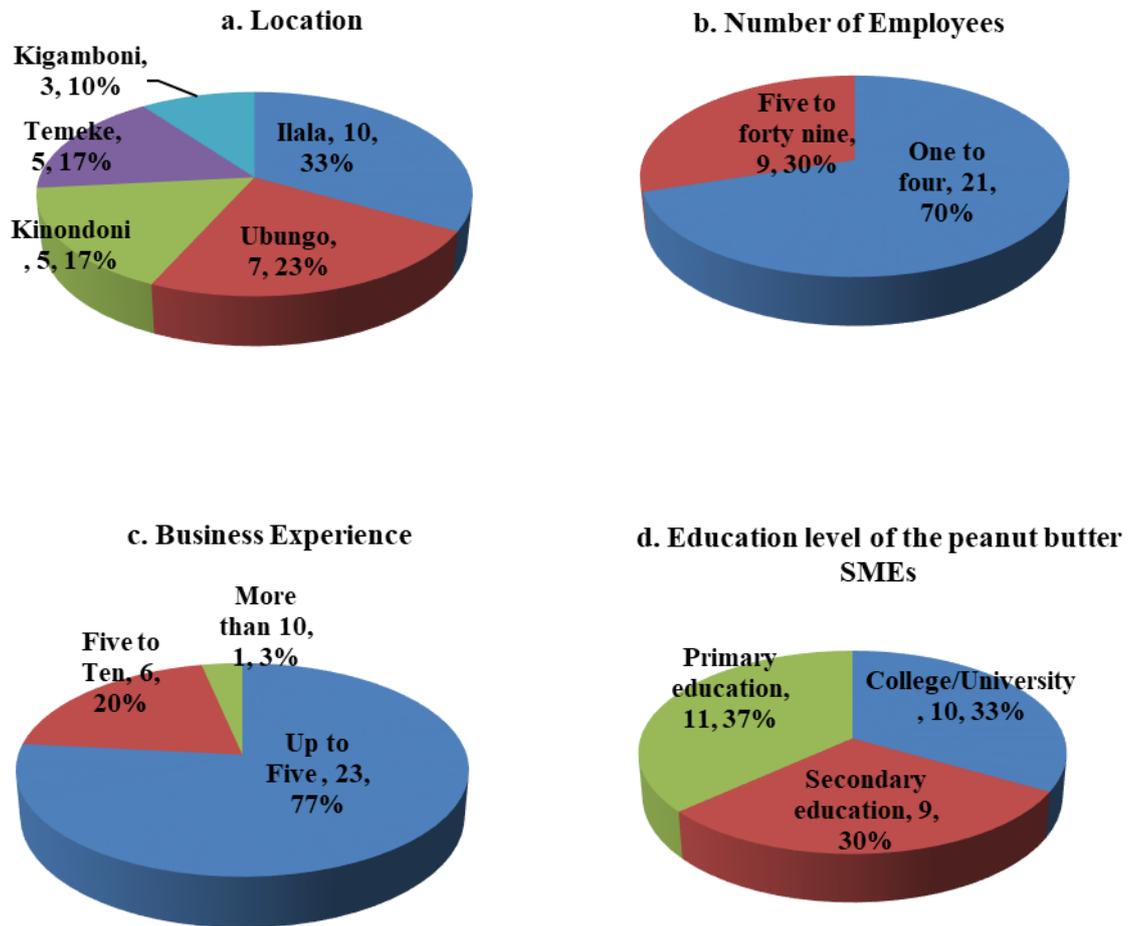


Figure 3 (a-d): Information of the peanut butter SMEs (N=30)

Seventy seven percent of peanut butter SMEs had less than 5 years of business experience in dealing with manufacturing of peanut butter while 3% of peanut butter SME had more than 10 years of business experience in dealing with manufacturing of peanut butter (Figure 3c). The education level of the peanut butter SMEs were 67% primary and secondary school education and 33% College/University education (Figure 3d). Similarly, Zinyemba and Changamire, (2014) in their study found that most of the SMEs lack higher education levels which justify their reason for them to engage in small businesses.

4.1.2 Contamination of peanut butter with aflatoxin

Seventy three percent of peanut butter collected were contaminated with AFB1, 63.3% contaminated with AFB2, 43.3% with AFG1 and 36.7% with AFG2 (Table 1). The total aflatoxin contamination was observed to be 76.7%. Ubungo municipality had highest number of contaminated samples with 85.7% AFB1 and 85.7% AFB2, while Kinondoni had the lowest number of contaminated samples of 40% AFB1 and 20% AFB2 (Table 1). Temeke had highest number of contaminated samples of AFG1 and AFG2 with 60%. Ubungo had higher 85.7% of contaminated samples of total aflatoxin. Furthermore, Kinondoni was observed to have lower 60% of contaminated samples of total aflatoxin (Table 1).

This results shows that there was variation in number of contaminated samples with different types of aflatoxins. This might be due to the quality of raw material which obtained from different local markets in Dar es Salaam region. Ndung'u *et al.* (2013) in their study on aflatoxin contamination in peanut and peanut butter samples collected from different local markets in Nairobi, found higher contamination of aflatoxin due to poor quality of raw material. The choice of raw materials is very crucial during peanut butter processing, as it contributes to the quality and safety of the final product. Matured and dry peanuts which having uniform size, and free from fungal contamination are recommended for peanut butter production (ITDG, 2002).

Table 1: Aflatoxin contamination of peanut butter from SMEs

Municipalities	AFB₁	AFB₂	AFG₁	AFG₂	Total AF
	n(%)	n(%)	n(%)	n(%)	n(%)
Temeke (N=5)	4(80.0)	4(80.0)	3(60.0)	3(60.0)	4(80.0)
Ilala (N=10)	8(80.0)	7(70.0)	4(40.0)	3(30.0)	8(80.0)
Kinondoni (N=5)	2(40.0)	1(20.0)	3(60.0)	2(40.0)	3(60.0)
Ubungo(N=7)	6(85.7)	6(85.7)	3(42.9)	3(42.9)	6(85.7)
Kigamboni(N=3)	2(66.7)	1(33.3)	0(0.0)	0(0.0)	2(66.7)
Total	22 (73.2)	19(63.3)	13(43.3)	11(36.7)	23(76.7)

N: is the total number of samples analysed in each municipality, n is the total number of contaminated samples

4.1.3 Levels of aflatoxin contamination in peanut butter

There were significant ($p < 0.05$) variations in aflatoxin levels between samples from different SMEs with AFB₁ ranging from 0.1 to 277.73 μ g/kg, AFB₂ ranging from 0.075 to 39 μ g/kg, AFG₁ ranging from 0.1 to 89.4 μ g/kg, AFG₂ ranging from 0.1 to 9.9 μ g/kg and total aflatoxin ranging from 0.1 to 317 μ g/kg (Table 2). Overall these results indicate that aflatoxin contamination levels in all thirty peanut butter samples ranged from 0.075 to 317 μ g/kg.

Moreover, the variations in aflatoxin levels between peanut butter samples were significantly different ($p < 0.05$) with sample PB 20 having highest AFB₁, AFB₂ and total aflatoxin levels of 277.7 μ g/kg , 39 μ g/kg and 317.0 μ g/kg respectively than other samples. Sample PB11 and sample PB14 had significantly highest AFG₁ and AFG₂ levels of 89.4 μ g/kg and 9.9 μ g/kg respectively than other samples some with values close to 0 μ g/kg (Table 2). Other studies have also reported variation in aflatoxin levels in peanut butter (Elzupir *et al.*, 2011; Mupunga *et al.*, 2014). Higher level of AFB₁ among peanut butter samples with an average concentration of 120 μ g/kg has been reported in Sudan (Elzupir and Alamer, 2014) while Boli *et al.* (2014) found lower levels of AFB₁

ranging from 0.23 to 2.49 $\mu\text{g}/\text{kg}$ in marketed peanut butter in Abidjan district, Ivory Coast.

Table 2: Aflatoxin contamination levels ($\mu\text{g}/\text{kg}$) in peanut butter samples among SMEs

Sample code	AFB1	AFB2	AFG1	AFG2	Total
PB1	nd	nd	nd	nd	nd
PB2	0.8±0.22 ^f	0.1± 0.01 ^g	nd	nd	0.8± 0.22 ^f
PB3	5.1±0.49 ^f	0.7±0.11 ^{efg}	1.9± 0.23 ^e	0.2±0.06 ^b	7.8 ± 0.88 ^{ef}
PB4	nd	nd	nd	nd	nd
PB5	nd	nd	nd	nd	nd
PB6	1.1± 0.13 ^f	0.1± 0.04 ^g	nd	nd	1.2± 0.18 ^f
PB7	nd	nd	0.4 ±0.06 ^e	0.2 ±0.01 ^b	0.6± 0.06 ^f
PB8	0.2±0.01 ^f	nd	nd	nd	0.2± 0.01 ^f
PB9	4.6±0.47 ^f	0.8±0.13 ^{efg}	nd	nd	5.2 ± 0.60 ^f
PB10	64.3±0.73 ^c	13.1± 0.21 ^c	26.1 ± 0.60 ^c	4.6 ±0.12 ^{ab}	108.1±1.65 ^c
PB11	100.3 ± 14.6 ^b	15.9± 2.11 ^b	89.4±12.4 ^a	6.6±9.38 ^{ab}	212.2± 19.78 ^b
PB12	nd	nd	nd	nd	nd
PB13	2.8±0.39 ^f	0.075± 0.06 ^g	0.4 ± 0.16 ^e	0.1±0.01 ^b	3.3 ± 0.62 ^f
PB14	53.3±1.94 ^c	10.7± 0.33 ^d	35.6± 0.57 ^b	9.9±0.08 ^a	109.5±1.63 ^c
PB15	20.3±1.15 ^d	2.1± 0.15 ^e	0.7± 0.05 ^e	0.2±0.01 ^b	23.3±1.34 ^e
PB16	nd	nd	nd	nd	nd
PB17	1.96±0.45 ^f	0.2 ±0.06 ^g	nd	nd	2.2 ± 0.50 ^f
PB18	1.6±0.057 ^f	0.1 ±0.18 ^g	nd	nd	1.8 ± 0.23 ^f
PB19	nd	nd	nd	nd	nd
PB20	277.7 ± 12.14 ^a	39.0± 0.57 ^a	0.3 ± 0.45 ^e	nd	317.0±12.26 ^a
PB21	4.2±0.51 ^f	0.4± 0.082 ^{fg}	2.8± 0.36 ^d	0.3 ±0.06 ^b	7.8±1.03 ^{ef}
PB22	0.6±0.01 ^f	0.01 ±0.00 ^g	0.4± 0.02 ^e	nd	1.0 ± 0.03 ^f
PB23	4.5±0.27 ^f	0.3 ±0.03 ^g	nd	nd	4.7± 0.30 ^f
PB24	9.8 ±0.56 ^{ef}	1.4± 0.11 ^{efg}	4.7 ± 0.53 ^d	0.3±0.06 ^b	16.3± 1.29 ^{ef}
PB25	0.1±0.14 ^f	nd	0.1±0.14 ^e	0.1± 0.00 ^b	0.3 ± 0.00 ^f
PB26	14.0±1.01 ^{ef}	1.5± 0.11 ^{efg}	nd	nd	15.5± 1.12 ^{ef}
PB27	9.5±0.56 ^{ef}	2.0± 0.11 ^{ef}	1.6± 0.10 ^d	0.1± 0.09 ^b	13.19± 0.86 ^{ef}
PB28	nd	nd	nd	nd	nd
PB29	31.6±1.01 ^d	13.9± 0.34 ^c	nd	nd	45.5± 1.34 ^d
PB30	0.1±0.11 ^f	nd	nd	nd	0.1± 0.11 ^f
TBS	5.000±0.00 ^F				15±0.00

Key: nd means not detected. Values are means ± standard deviation of the mean of duplicate determinations. Values in the same column having different superscript letters are statistically different at $p < 0.05$.

4.1.4 Total aflatoxin and AFBI levels in peanut butter samples to TBS regulatory limits

Figure 4 compares AFB1 and total aflatoxin levels to the limits set by TBS as stipulated in TZS 844:2014. About 45.5% samples were contaminated with AFB1 and 37.5% samples were contaminated with total aflatoxin above the TBS regulatory limits for 76.7% of the samples that contaminated with aflatoxins. This means that, 33.3% samples were contaminated with AFB1 and 26.6% samples were contaminated with total aflatoxin above the TBS regulatory limits for all thirty peanut butter samples.

This result reveals that few samples were contaminated with AFB1 and total aflatoxin above the TBS regulatory limit for all thirty peanut butter samples. This might be due to the fact that, peanut butter SMEs were aware on favorable conditions for aflatoxin occurrence in peanut for peanut butter production. However, occurrence of the aflatoxin above the limit might be due to poor practice observed during survey of temporarily storing crushed peanuts in plastic bucket for some time (up to a month) before grinding into the machine. Similarly, Elzupir *et al.* (2011) observed in Sudan that in all surveyed factories the peanuts were crushed and kept in plastic bags at ambient temperature before butter making. Effect of high and favorable temperature condition for growth of aflatoxin-producing fungi was reported by the study conducted in Sudan in which 90% of the peanut butter samples exceeded the European Union maximum limit of 20 µg/kg (Elzupir *et al.*, 2011). Occurrence of AFB1 above the allowable limits in food products is a common problem to many developing countries as it was also reported in a survey conducted in Zimbabwe that all of the peanut butter samples were contaminated at levels that exceed the maximum AFB1 level set by Zimbabwean legislation (5 µg/kg) in all foods (Mupunga *et al.*, 2014).

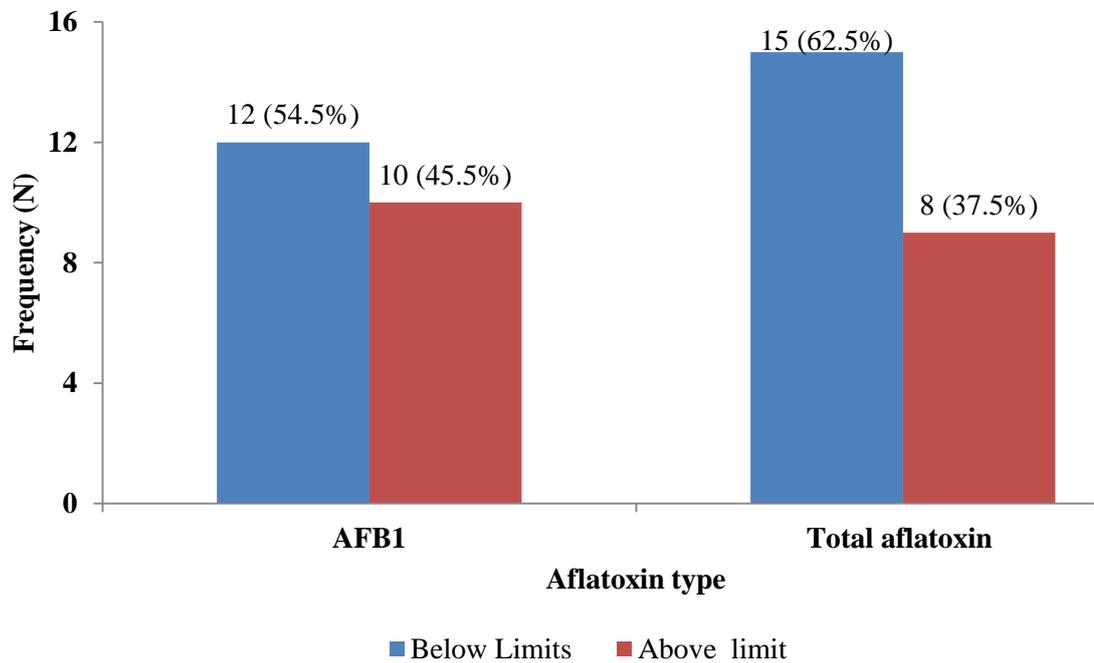


Figure 4: Comparison of total aflatoxin and AFB1 levels in peanut butter samples with TBS limits

4.2. SMEs Awareness on Aflatoxin Contamination in Peanut Butter

4.2.1 Source of peanut, criteria and indicators used to monitor peanuts from aflatoxin contamination

Fifty seven percent of peanut butter SMEs obtained their raw material from local markets in Dar es Salaam region while 26.7% and 16.6% obtained their raw material direct from the farm in other regions of Tanzania, and both (from the market and direct from the farm) respectively (Table 3).

The result shows that all peanut butter SMEs had the criteria (acceptance /rejection) for receiving peanuts for peanut butter production and used visual quality check (color, aroma, size, insect damage, etc.) as an indicator to monitor peanuts from aflatoxin contamination. Similarly, PACA, (2016) reported that most of the peanut producers used visual methods to check the quality of peanuts for aflatoxin control. According to Park, (2002) sorting out physically damaged and infected grains from produce can result in 40 to 80% reduction in aflatoxin levels.

Table 3: Source of raw materials, criteria for receiving raw materials and indicator for monitoring peanut contamination

Variable	Category	Frequency	Percent
Source of peanuts	Direct from Farmers	8	26.7
	Market	17	56.7
	Both	5	16.6
	Total	30	100
Availability of criteria for receiving peanuts	Yes	30	100
	No	0	0.0
	Total	30	100
Indicator used to monitor Peanut contamination	Visual quality check	30	100
	Other indicator/s	0	0.0
	Total	30	100

4.2.2 Equipment used to store peanuts

The result shows that 53.3% of peanut butter SMEs in Dar es Salaam region commonly use plastic buckets while 46.7% use polypropylene bags, to store peanuts for peanut butter production (Figure 5). However, jute bags (sisal bags) are recommended for storage as plastic bucket, polypropylene and polyethylene bags could attributed to retention of heat and moisture which promote the fungal growth (Mutegi *et al.*, 2013).

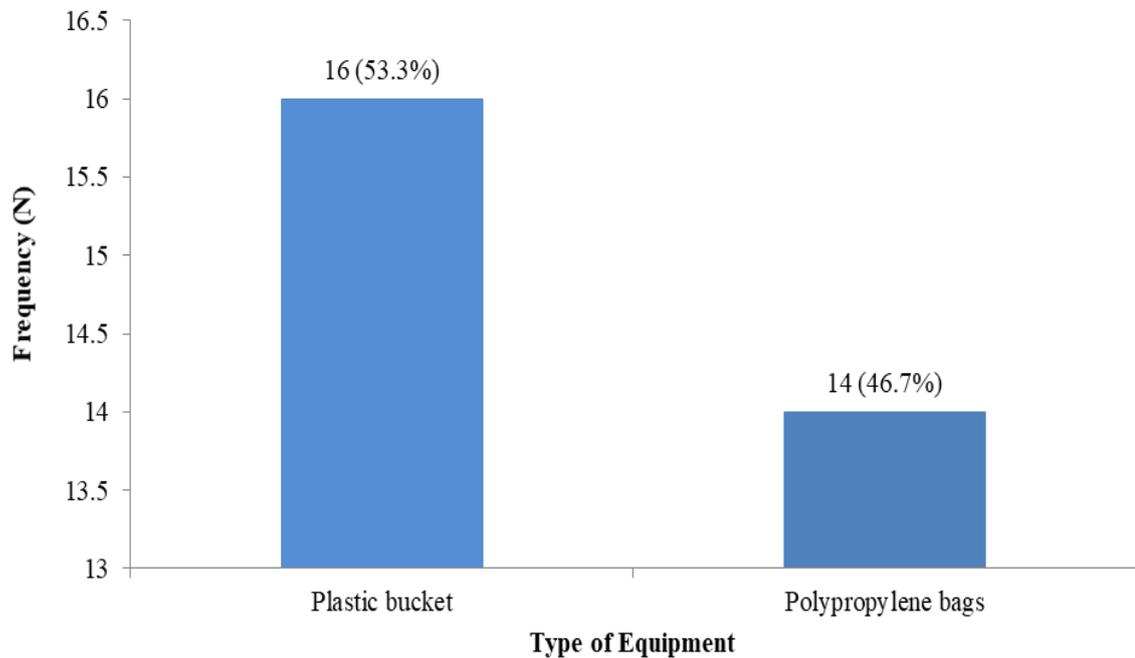


Figure 5: Type of equipment used to store peanut by peanut butter SMEs

4.2.3 Knowledge and source of knowledge of peanut butter SMEs on aflatoxin contamination

Knowledge and training are the most influential factors towards minimizing aflatoxins contamination in peanut based products including peanut butter (Azaman *et al.*, 2016). The current study observed that all peanut butter SMEs had knowledge on susceptibility of peanut and peanut butter to aflatoxin. About 96.7% of peanut butter SMEs were aware on favorable conditions for aflatoxin in peanuts while 3.3% peanut butter SME was not aware (Table 4). Furthermore, those peanut butter SMEs who were aware on aflatoxin contamination mentioned high moisture peanuts, high storage temperature, and peanuts stored for a long time as the conditions for aflatoxin occurrence in peanuts which was also reported by other studies (Kana *et al.*, 2013; Villers, 2014).

The result showed that majority 73.3% of peanut butter SMEs was trained by SIDO on Aflatoxin contamination while 16.7%, 6.7% and 3.3% of peanut butter SMEs learned from the media, college/university and TFDA, respectively (Table 4). These findings justify what has been observed during survey whereby most of these peanut butter SMEs were knowledgeable enough on aflatoxin contamination in peanut and peanut butter.

Table 4: Knowledge of SMEs and their sources of knowledge

Knowledge and Source	Category	Frequency	Percent
Knowledge on susceptibility of peanut and peanut butter to aflatoxin	Yes	30	100
	No	0	0.0
	Total	30	100
Knowledge on favorable conditions for aflatoxin in peanuts	Not aware	1	3.3
	aware	29	96.7
	Total	30	100
Source of knowledge	College/University	2	6.7
	Media	5	16.7
	SIDO	22	73.3
	TFDA	1	3.3
	Total	30	100

4.2.4. Quality and safety peanut butter

About 83% of peanut butter samples were not submitted to food regulatory authorities for aflatoxin analysis as required by Food registration regulation of 2011 under TFDA (Figure 6a). Only 16.7% of peanut butter samples were tested for levels of aflatoxin contamination of which the test was done for the purpose of being registered by food regulatory authorities and or to obtain the product TBS mark. Whereas, after fully registered other batches of peanut butter were not submitted for testing aflatoxin level.

The results showed that cost for aflatoxin analysis was a major challenge among peanut butter SMEs. Result was reported by Mutegi *et al.* (2013) that majority of the food manufacturers in Kenya failed to test their food products including peanut butter due to high cost for aflatoxin test.

This study also observed that, among 30 premises of peanut butter production which were visited during the survey, about 85% of supervisors were available in ensuring quality and safety (Figure 6b). However 53% of supervisors attended training in quality and safety while 47% did not attend the training (Figure 6c). During the survey, it was observed that these peanut butter SMEs as the owner of the premise also served as the supervisor in ensuring quality and safety of the product. Whereas training on quality and safety of the food products including peanut butter was obtained from SIDO on GMP and GHP. The experience gained from SIDO has shown that 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). Food safety training is associated with improved knowledge among food handlers and deliverers of the food which is safe from contamination (Adesokan *et al.*, 2015).

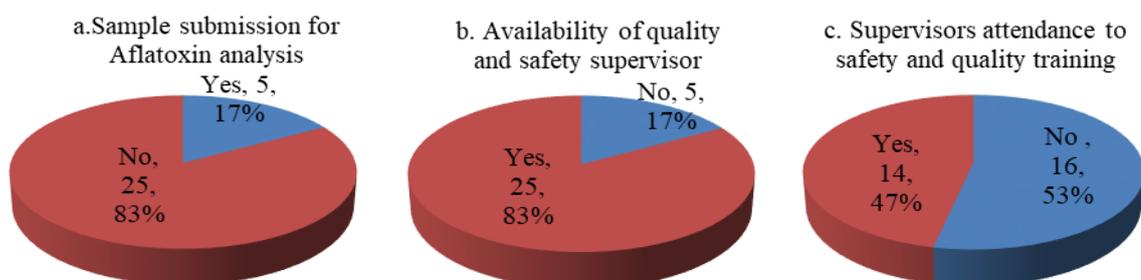


Figure 6 (a-c): Safety and quality consideration in peanut butter SME

4.3 Compliance of Peanut Butter SMEs with National Food Laws

Results in Table 5 showed that, majority 96.7% of peanut butter SMEs had their processing place in clean condition of which only 26.7% of peanut butter SMEs stored the raw material (peanut) on pallets. Nevertheless, majority 76.7% of peanut butter SMEs did not have a premise that met the requirements set by TFDA for peanut butter production. The present study found that lack of financial capacity to these peanut butter SMEs was a reason for not affording to build a food premise that meet the set requirements. This finding was also reported by Hasnan *et al.* (2014) that lack of financial resources was the main challenge faced SMEs of which influence their capability to design and build a required food production premises. This situation justifies observation in this study that majority 80% of peanut butter SMEs their premise were not registered and uses single room to perform all the production processes. Hasnan *et al.* (2014) reported that poor design of food production premise may lead to unsafe food products which could be among the reasons for aflatoxin contamination in most peanut butter samples collected in this result.

Moreover, the results showed that 20% of peanut butter SMEs had registered their peanut butter production premise as required by TFDA, 16.7% registered their products, 10% their products had TBS mark and most 56% of peanut butter SMEs operates their business without business license (Table 5). This finding is in line with observation made by Nyamwanza *et al.* (2014) who reported that many SMEs are not registered and they operate informally. Yapp and Fairman (2006) recommended that compliance with food laws is a continual process which requires monitoring and evaluation to ensure adherence to the required food safety standards by SMEs in order to avoid contamination of food products to include aflatoxin.

Table 5: Compliance of SMEs with regulatory requirements

Variable	Category	Frequency	Percent
Premise meet requirements from food regulatory authorities	Yes	7	23.3
	No	23	76.7
	Total	30	100
Peanut stored on pallets	Yes	8	26.7
	No	22	73.3
	Total	30	100
Processing place in clean condition	Yes	29	96.7
	No	1	3.3
	Total	30	100
Product has TBS Mark	Yes	3	10.0
	No	27	90.0
	Total	30	100
Premise has registration certificate	Yes	6	20.0
	No	24	80.0
	Total	30	100
Product has registration certificate	Yes	5	16.7
	No	25	83.3
	Total	30	100
Presence of business license	Yes	13	43.3
	No	17	56.7
	Total	30	100

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

This study revealed that most 76.7% of the peanut butter samples from selected SMEs were contaminated with aflatoxins AFB1, AFB2, AFG1 and AFG2. Some of the peanut butter samples had high concentration of aflatoxin level which poses significant threats to the health of consumers. In line with the TBS regulatory limits, 33.3% and 26.6% of the total peanut butter samples exceeded the limit for AFB1 and total aflatoxin, respectively. This lower level of aflatoxin contamination above the TBS limit was attributed by the fact that most of the peanut butter SMEs had awareness on the aflatoxin contamination in peanut butter. However, this study found that most of the peanut butter SMEs did not register 80% of their premises and 83.3% of products and also 90% of the products did not have TBS marks. Moreover, some 56.7% of peanut butter SMEs did not have business license.

5.2 Recommendations

Recommendations for improvement in reducing aflatoxin contamination, awareness and compliance of peanut butter SMEs from established food laws are outlined below:-

- i. Analysis of peanut butter samples from each batch before reaching consumers should be made mandatory in order to confirm that levels of aflatoxin contamination are within safety limits.
- ii. Costs for aflatoxin laboratory test should be made reasonable to encourage peanut butter SMEs to test their product before making them available to consumers.

- iii. Continuous training programs and education with regards to aflatoxin contamination should be conducted to peanut butter SMEs in order to improve their awareness on favorable conditions/environment for aflatoxin occurrences, and knowledge on food safety so as to produce a peanut butter which is safe from aflatoxin contamination.
- iv. SMEs peanut butter manufacturers should be trained on effective ways of minimizing aflatoxin contamination, such as sorting peanuts.
- v. Peanut butter SMEs should improve their working place (premise) as recommended by regulatory authorities so as to produce peanuts that are safe for human consumption.
- vi. Food regulatory authorities should make an effort to identify all peanut butter SMEs and location of their working place (premise) to ensure their products and premise are registered and certified.
- vii. Follow up inspection should be made mandatory for the SMEs peanut butter manufacturers with effective monitoring to make sure that products are always produced in a safe environment to minimize aflatoxin contamination and to ensure compliance and legal measures are taken to non-complying peanut butter SMEs.
- viii. Further studies need to be conducted on aflatoxin contamination which involves peanut butter samples with reference samples.

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APPENDICES

Appendix 1: Semi-structured questionnaire for SMEs peanut butter manufacturers

Introduction

My name is Lulu Kiwia, a student from Sokoine University of Agriculture. I am currently conducting my research on determination of the Aflatoxin in Peanut butter and Compliance to National Food Laws by Small and Medium Manufacturers in Dar es Salaam region. This study is for attaining my MSc. Food Quality and Safety Assurance. This questionnaire applies to all selected small and medium manufacturers of Peanut butter in Dar es Salaam region. It entails at obtaining more information on awareness of Aflatoxin contamination. Kindly, you are asked to respond to questions in this questionnaire and information which you are going to provide will be treated as confidential and used to facilitate the intended learning at the Sokoine University of Agriculture and not otherwise.

General Information:

1. Manufacturers Name.....Code No:
2. Number of employees.....
3. Date of Interview.....
4. Sex.....
5. Municipality.....
6. Business experience
 - a. Up to 5 years b. 6 – 10 years c. More than 10 years
7. Education level attended
 - a. College/University b. Secondary education c. Primary education

Awareness on Aflatoxin in Peanut butter

Source of peanuts, Criteria and indicators to monitor peanuts from aflatoxin contamination

1. Where do you source peanuts for peanut butter production?
Market [], direct from the farm [], others (Specify)
2. Do you have criteria (acceptance/rejection) for receiving the peanuts (Yes/No)?
3. If Yes, what indicator(s) do you use to monitor Peanuts from Aflatoxin contamination? (Tick) a. Visual quality check (color, aroma, size, insect damage, etc) [] b. Storage Temperature [] c. Laboratory Test [] d. [] No monitoring

Equipment used to store peanuts

1. What package do you use to store peanuts?

Knowledge of SMEs and their source of knowledge

1. Do you know that Peanuts are susceptible to Aflatoxin contamination? Yes/No (Tick one)
2. If Yes, where did you learn about Aflatoxin contamination in Peanuts? (Tick)
a. College/University [] b. VETA [] c. TFDA [] d. Media []
e. SIDO f. Others (Specify).....
3. Do you know the favorable conditions/environment for Aflatoxin occurrence in Peanuts? Yes/No, If Yes, explain briefly
.....
.....

Quality and safety considerations

1. Did you submit the sample of peanut butter to TFDA/TBS for Aflatoxin analysis?
(Yes/No)
2. Does your factory have a supervisor to supervise and ensure safety and quality of Peanut butter production? (Yes/No)
3. Supervisor attendance to quality and safety training (Yes/No)
4. What challenges are you facing with regards to compliance to legal requirements and ascertaining and controlling aflatoxin contamination in peanut butter?

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THANK YOU

Appendix 2: Observation checklist for SMEs peanut butter manufacturers

Manufacturer Name:

Code No: -----

Municipality: -----

NA	ITEM	YES	NO	REMARKS
1	The Premise meet requirements from TFDA/TBS			
2	Peanuts are stored in a clean and dry environment			
3	Peanuts stored on pallet			
4	The product has TBS mark			
5	The premise has a TFDA premise registration certificates			
6	Product has TFDA registration certificate			
7	Business license			