FUMONISIN CONTAMINATION IN CEREAL-LEGUME COMPLEMENTARY FLOUR MARKETED IN DAR-ES-SALAAM

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HUMAN NUTRITION OF SOKOINE UNIVERSITY OF AGRICULTURE.

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ABSTRACT

Surveillance was conducted during the year 2016/2017 in Dar es Salaam region, Tanzania to determine the levels of fumonisins contamination in pre-packed cereal-legume complementary flour. The concentration of fumonisin was determined using high-performance liquid chromatography (HPLC). The concentration of fumonisin B_1 ranged from 2. to 60 669µg/kg (mean 2371.8 µg/kg), fumonisin B_2 ranged from 5.74 to 15 758 µg/kg (mean 901.2 µg/kg) and the total fumonisins (FB_1 + FB_2) ranged from 8.2 to 61 486.8 µg/kg (mean 3 273.0 µg/kg). The levels of fumonisin B_1 and total fumonisins exceeded the maximum limit of 2000 µg/kg for processed cereals and products intended for human consumption. There was significant difference ($p<0.05$) between the levels of fumonisin contamination and type of packaging material. The flour packed in boxes and plastic was more contaminated and levels exceeded acceptable tolerable limit. The fumonisin exposure to children through cereal-legume complementary flour was estimated. The estimate from the study shows the fumonisins contamination in cereal-legume complementary flour and children were exposed to fumonisins above provisional maximum tolerable daily intake PMTDI through consumption of the contaminated complementary flour, which has negative effect on human health. Therefore appropriate practices recommended along the commodity value chain that can reduce contamination in order to improve food safety and food security.

DECLARATION

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

_____ _____

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(MSc. Human Nutrition)

The above declaration is confirmed;

_____ _____

Prof. Jovin K. Mugula Date

(Supervisor)

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DEDICATION

I would like to dedicate this work to my husband Abdallah Mussa, our lovely children Munir and Malha, my parents for their prayers, love, sacrifice and patience during the entire period of my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

EAC	East African Community
EU	European Union
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FAO	Food and Agriculture Organization
HPLC	High Performance Liquid Chromatography
iAGRI	Innovative Agricultural Research Initiative
IARC	International Agency for Research on Cancer
IITA	International Institute of Tropical Agriculture
LOD	Limit of Detection
LSMeans	Least Square Means
MATI	Ministry of Agriculture Training Institute
MC	Moisture content
µg/kg	Microgram per Kilogram
Mg/kg	Milligram per Kilogram
MTL	Maximum Tolerable Limits
NM-AIST	Nelson Mandela African Institute of Science and Technology
NTD	Neural Tube Defect
PMTDI	Provisional Maximum Tolerable Daily Intake
PACA	Partnership for Aflatoxin Control in Africa
PBS	Phosphate Buffered Saline
SAX	Strong-Anion-Exchange Columns

SD	Standard Deviation
SE	Standard Error
SUA	Sokoine University of Agriculture
TFDA	Tanzania Food and Drugs Authority
USA	United States of America
USAID	United States Agency for International Development
UV	Ultra Violet
WEMA	Water Efficient Maize for Africa

CHAPTER ONE

1.0 INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi and contaminate food and feeds (IARC, 2002; Wu *et al.*, 2014). These toxic metabolites commonly occur in cereal- and cereal-based products (Bhat *et al*., 2010). The most common effect of mycotoxins exposure to human health, referred to as mycotoxicoses, may appear as a disease or a physiological disorder (Wild and Gong, 2009). Most of the mycotoxins are teratogenic, mutagenic and carcinogenic to animals and humans (Mohammad *et al.,* 2012).

The predominant mould strains that produce mycotoxins are *Aspergillus*, *Fusarium* and *Penicillium* species (Bennett and Klich, 2003). *Fusarium* is one of the most important genera of phytopathogenic fungi that can infect cereal grains and produce mycotoxins accumulation (Soler, 2013). The known economically important mycotoxins produced by *Fusarium* species are fumonisins, zearalenone and trichothecenes (T_2, TH_2 and deoxynivalenol) (Darwish *et al.*, 2014), which are widely distributed in maize products and are associated with many ill-health hazards to human and animals (Fandohan *et al.*, 2003). Fumonisins are among the groups of mycotoxins with a wide geographical distribution and present in many parts of the world that grow maize (Marasas *et al.*, 1996; Devide *et al.*, 2016). The distribution of fumonisins in the world depends not only on the environmental conditions but also on exogenogous factors that affect both *Fusarium* population as well as mycotoxin production (Devide *et al.*, 2016). Fumonisins are produced by *Fusarium* species mainly are *F verticillioides, F. proliferatum* and F. *nyagmai.*

Fumonisins were first identified by Gelderblom *et al.* (1988) under three major groups, namely fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3). The FB_1 and

FB_2 have toxicological significance to human and animal species while FB_3 occurs at very low concentration and become less toxic (Perraica, 1999; Nyangi *et al.*, 2016). The FB_1 and FB_2 are most abundant in maize than other cereal crops, and contaminate the maize crop in the field prior to harvest (Lombard, 2014). Apart from maize and maize products, fumonisins contaminate other grains such as sorghum, oats, wheat and beans (Mamiro, 2005; Morgensen *et al*., 2010). Fumonisins contamination in most substrates is highly dependent on the composition of the *Fusarium species* in the area as well as environmental conditions. Studies on occurrence of fumonisins in food and feed indicates that water stress during drought period is associated with *Fusarium* infection particularly *F*. *verticillioides.* Under such condition, the fungi grow well and produce toxins that accumulate in maize kernels (Miller *et al.*, 2001; Nosrat, 2015). Climatic conditions such as rainfall and humidity in different areas affect the incidence of different Fusarium species responsible for production of other mycotoxins in the cereals (Osborne *et al.*, 2007; Devide *et al.*, 2016).

The naturally occurring fumonisins in foods and feeds are FB_1, FB_2 and FB_3 (Ncube *et al.*, 2011). The FB_1 and FB_2 are pathologically referred as potent toxicological compounds occurring in plant-based foods that interfere with metabolism in human cells and play role in the occurrence of oesophageal cancer. Available studies demonstrate persistent exposure to fumonisins as a risk to high incidence of oesophageal cancer in rural and peri-urban communities where fumonisin contamination in maize was prevalent such as the former Transkei region of South Africa (Xu *et al.*, 2010); Shirima *et al.*, 2015) and Iran (Mohammad *et al.*, 2012).

Liver cancer is other toxicological effects from prolonged consumption of food contaminated with fumonisins especially B_1 and B_2 (IARC, 2002). Contamination of food

or feed with fumonisins and exposure varies with geographical location, for instance in part of China when a person is exposed to food contaminated with FB_1 was considered as group 2B carcinogens (IARC, 2002). Exposures to Fumonisins were also reported to interfere with sphingolipids metabolism (Wild, 2009; Martin e*t al*., 2010) that impairs child growth (stunting and underweight) and neural tube defect (Kimanya *et al*., 2010). Recent studies reported that the consumption of the food that contains high contamination of fumonisins of FB_1 above the PMTDI was associated with human oesophageal cancer (Rheeder *et al*, 1992), growth retardation in children under two years of age (Kimanya *et al*., 2010), and increase in the incidence of neural tube defects as it interfere with sphingolipids metabolism and folic acid absorption (Bordin *et al.*, 2015). Zearalenone, Deoxynivalenol and Trichothecenes are other mycotoxins produced by *Fusarium* species which contaminate maize and wheat products that used as food or feed. ; They suppress immunity and enlargement of the mammary gland causing ill-health effects due to cumulative toxicity when exposed for a prolonged period (WHO, 1999; Devide *et al.*, 2016). The trichothecenes (deoxynivalenol and patulin) inhibit protein synthesis, cause nausea, vomiting and diarrhoea. They are vomitoxins and immunotoxic that affect changes in brains cells neuro-chemicals (Devide *et al.*, 2016).

Cereals such as maize, rice, wheat, millets and sorghum are most preferred staple foods in sub-Saharan countries; where they account for 75% of consumption and crop production (WEMA, 2010). Maize is one of the main cereals used as staple food in Tanzania as well as composite flour used as complementary foods (Suleiman, 2015). Other cereals used include sorghum, rice, wheat and finger millet as they contain high concentrations of energy, sources of carbohydrates, proteins, fat and micronutrients (Shirima *et al.*, 2013 Kana *et al.*, 2013 and Gheysens, 2015). Complementary foods are semi-solid foods that are given to children when breast milk alone is insufficient to meet the nutritional

requirements for children aged 6 to 59 months (WHO, 1999; Muhimbula *et al.*, 2010). Cereal-legume complementary foods are used as major sources of carbohydrate, fibres and energy for children under five years of age in developing countries (Abeshu *et al*., 2016).

Most of the cereal-legume complementary flour is packaged in different types of packaging materials. Food packaging is a coordinated system for preparing and containment for better distribution, storage and satisfying customer needs. Packaging is essential in protection of processed and unprocessed food against contamination and waste due to spoilage (Shin and Selke, 2010). Materials that are available for packaging cereal flour are polyethylene (nylon), paper and paper based material, boxboard and aluminium. They are used to control moisture content in foods, temperature and insect infestation which have an effect on fumonisins contamination (John and Antonio, 2014).

Conditions such as like moisture content, temperature, air circulation and mixture of the cereal-legume composite flour during processing and packaging have impact on mycotoxins production (Mohammad *et al.,* 2012). The type of packaging material and storage practices could create the condition that allows growth of mould responsible for the production of mycotoxins (Mutegi *et al.,* 2013).: it is reported the increased moisture, carbon dioxide and temperature during processing and packaging may favor the growth of mycotoxins especially aflatoxins in stored cereal flour (Mutegi *et al.,* 2013).

1.1 Justification

Mycotoxins contaminate cereal crops especially maize from the field to the post-harvest storage (Atanda *et al*., 2013). The incidence of fumonisin contamination in homemade

stored maize and cereal-based complementary food was observed to be high in Tanzania up to (11.048mg/kg) and (2.283mg/kg), respectively (Kimanya *et al*., 2008, 2010). Human exposure to mycotoxins has raised worldwide concerns due to their health impacts in humans and animals (IARC, 2002). The fumonisins produced by the *F. vertilliocides* are dominant in African countries, and are associated with human oesophageal cancer and cardiovascular diseases (Morgensen *et al.,* 2011). Human exposure to fumonisins is associated with increased susceptibility to HIV infection due to impaired immunity (Williams *et al*., 2010). The exposure to fumonisins in early stage of life could be detrimental due to the child's immature liver for detoxification and could impair their growth and suppresses immunity (Norhasima *et al.,* 2009). Other ecological study conducted in other countries correlates the high incidence of human oesophageal cancer and neural tube defects with exposure to fumonisins in South Africa (Rheeder *et al.,* 1992; Shephard *et al.,* 2007), Asian countries (Finchan *et al.,* 1992) and Northern American countries (Melorose *et al.,* 2015). In Tanzania other study conducted demonstrate chronically exposure of children to aflatoxins and fumonisins in rural areas of Tanzania due to consumption of contaminated maize-based complementary foods and has been associated with linear growth retardation among young children aged 6-8 month's (Kimanya *et al*., 2010; Shirima *et al*., 2013).

Dietary exposure to fumonisins in young children determined using validated biomarkers of exposure indicated that children in selected villages in Tabora, Kilimanjaro and Iringa were chronically exposed to aflatoxins and fumonisins due to consumption of contaminated maize and maize-based complementary foods (Kimanya *et al*., 2009; Shirima *et al*., 2015). The risk of exposure to mycotoxins among children was found to increase with age as the amount of food consumption increases (Magoha *et al.,* 2014).

Occurrence and co-occurrence of aflatoxins and fumonisins in maize and maize products as well as exposure to the mycotoxins has been reported in various region of Tanzania (Kimanya *et al*., 2009, 2010 and Nyangi *et al*., 2016). Kamala *et al.* (2016) reported the likelihood of association between the post-harvest practices and fumonisin contamination in maize and the contamination was more in unsorted and maize dried on the bare ground than in the sorted maize dried on mats.

Climate has an influence on occurrence of fumonisins in cereal crops and even if the weather conditions such as temperature, humidity and rainfall are favourable, the absence of appropriate management strategies of grains in the field influences the level of fumonisins contamination to the extent that the levels exceed the maximum tolerable limits (Devide *et al.*, 2016). The inherent moisture content, extent of air exclusion during packaging and presence of mould spores are important parameters that require attention prior to grain milling and packaging of complementary flours (Shakerardekani and Karim, 2013). Formulated complementary foods for older infants and young children should be packaged in materials which safeguard the hygienic and other qualities of the food. Packaging materials should be made from materials which are safe and suitable for their intended uses. They should also protect the contents against moisture absorption and production of fumonisins which have effect on safety and quality of the product (Shakerardekani and Karim 2013). The food safety aspects are important in ensuring food security, public healthy, economic growth as well as the improvement of nutrition status of children.

The aim of this study was to carry out surveillance on the levels of fumonisins contamination in the marketed cereal-legume based composite flours packaged in various

packaging materials and establish the exposure risk of children to the mycotoxin. This information might be important in designing intervention strategies on prevention and management of health risks associated with fumonisin contamination in cereal-legume composite flour.

1.2 Objectives

1.2.1 Overall objective

The overall objective of the study was to determine levels of fumonisin contamination in cereal-legume based complementary flours and to estimate levels of exposure of children under-five years of age to the toxin

1.2.2 Specific objectives

i. To determine levels of fumonisins B_1 and B_2 in cereal-legume complementary flours.
ii. To assess the association between type of packaging material and levels of fumonisin contamination in cereal-legume-complementary flour.
iii. To estimate level of exposure to fumonisins from cereal-legume complementary flour among children under five years of age.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Occurrence of Fumonisins

Fumonisins are very potent mycotoxins produced by *F. monoliforme* (*F. verticulliodes), F. proliferatum, F.nygamai* and *Alternaria alternata* mainly in maize all over the world. Cereals, legumes and some nuts are major crops attacked by *Fusarium* species (Marin *et al.,* 2013). Fumonisins are the cancer-promoting metabolites of *F. moniliforme* that have long chain hydrogen carbon unit which play roles in its toxicity (Zain, 2011). *Fusarium* species produces fumonisins B_1, $B_{2,}$ B_3 and $B_{4;}$ of which FB_1 and FB_2 are the most potent toxins and common pathogenic fungi that contaminated maize and maize products during pre-and post-harvesting period (Kumi *et al*., 2014). The *F. verticilliodes* is prevalent in warmer areas with high humidity and temperature above 28^{o}C, while *F.proliferatum* inhabit relatively in cooler areas (Gheysens, 2015). The F. *verticilliodes* infection and fumonisins accumulation predominantly occurs in maize kernel in warmer and drier climate while the *F. proliferatum* develop better in cooler and wetter climate (Knudsen *et al.*, 2011).

Increase in levels of carbon dioxide gas (CO_2) due to climate change that accompanied with increase in temperature weaken plants immunity; while favours the growth of *F.verticilliodes* in plants and increases the fumonisins production and contamination in agricultural food crops (Wu *et al*., 2011). *Fusarium* species that produce fumonisins grow on maize kernel in the field prior to harvesting and the occurrence of fumonisins is accompanied with other mycotoxins like aflatoxins and deoxynivalenol (DON) (Kimanya *et al.*, 2014). The occurrence and contamination of fumonisins in the agricultural products are very complex under field condition due to interaction of high temperature, water

activity, altitudes and seasonal variations. Climatic variability such as dryness and wetness of growing location influenced by temperature and water activity during kernel drying may trigger fumonisins biosynthesis in the weakened plant due to moisture stress (Santiago *et al.,* 2015). The increase in temperature from 1-3 ^{0}C predicts were predicted to increase in agricultural production, although the increase in temperature compromises food safety due to increased microbial contamination (Wu *et al.*, 2011).

2.2 Prevalence of Fumonisins Contamination in Food

The mycotoxins are known to contaminate cereals, legumes, nuts and spices all over the word, of which aflatoxins, fumonisins, zearalenone, ochratoxins and deoxynivalenol are some of the most prevalent (State, 2014). Mycotoxin contamination in cereals is potential risk to human and animal health. Among well-known groups of mycotoxin produced by *Fusarium species* are fumonisins, deoxynivalenol and zearalenone which are most common in food and feedstuffs in different parts of the world (Santiago *et al*., 2015). Over 14 types of mycotoxins were studied from Northern America, Asia and Africa in different cereals where it was revealed that over 53% were contaminated with different types of mycotoxins (Darwish *et al.,* 2014). Several studies have been conducted to determine the levels of fumonisins contamination in maize in different countries. According to Scussel *et al (*2014) the prevalence was 46.6% from 232 collected samples of maize in Brazil; in Croatia the prevalence was 90% from 63 collected maize samples with 1.67% samples exceeding maximum limit (Pleadin *et al.,* 2013). In Egypt the prevalence was 100 out of 20 maize samples collected (Nooh *et al.,* 2014). In Poland from 30 samples of maize collected all of them (100%) were contaminated (Czebor *et al.,* 2015). Similarly the prevalence was 30% with about 16.67% of samples containing fumonisin at levels exceeding the maximum limits in maize production areas especially in warmer areas such as Northern Cape, North West and Free State Provinces of South

Africa (Van Rensburg *et al.,* 2015). High prevalence of fumonisins accounted for about 76% of the analyzed maize samples, with an average level of 1501µg kg^{-1}(Sanders *et al.,* 2014).

In the year 2013, the prevalence of fumonisins in Asia was 52% and the highest level was found in South Asia (Anukul *et al.,* 2013). In African countries, the incidences of mycotoxins contamination were also recorded. The level of aflatoxins accounted for 43.5%, followed by fumonisins contamination, that accounted for 21.87%; others were ochratoxins, 12.5%; zearalenone, 9.375%; deoxynivalenol and beavericin, 6.25% of samples collected (Darwish *et al.,* 2014). *F. verticillioides* can constitute up to 95% of all *Fusarium* strains recovered from maize fields in African countries and produces secondary metabolites recognized as the cancer promoting (Ncube *et al.,* 2011). In Uganda, the overall contamination levels for total fumonisins in pre-harvest maize from different climatic zones ranged from 0.27 to10 mg/kg (Atukwase *et al.,* 2009). Similar results were also reported by Kaaya *et al.* (2006). In South Africa, fumonisins contamination of maize ranged from 0 to 21.8 mg/kg (Ncube *et al.,* 2011). In Kenya, where the largest known aflatoxicosis outbreaks occurred in year 2004, with 317 reported cases and 125 deaths (Probst *et al.*, 2007), 86 stored maize samples had mean fumonisins contamination ranging from 0.912 mg/kg to 1.17 mg/kg (Bii *et al.*, 2012).

In Tanzania the incidence of fumonisin exposure is higher in rural community due to their reliance on maize as staple food (Kimanya *et al.*, 2008). Over 12% of infants in rural areas of Tanzania were reported to exceed the provisional maximum tolerable daily intake from contaminated complementary foods (Kimanya *et al.,* 2010). The occurrence and levels of mycotoxins varies within a geographical regions, season and storage condition (Kimanya *et al.,* 2008). The occurrence of mycotoxins contamination showed likelihood

of association between the post-harvest practices and fumonisin contamination was more on the unsorted and dried maize on the bare ground than the sorted dried on mats as reported by (Kamala *et al.,* 2016).

Studies conducted in Manyara region of Tanzania, indicated that the incidence of fumonisins contamination in marketed maize was higher (5µg/kg) in the highland zones of the regions which received high amount of rainfall than to aflatoxins (Nyangi *et al*., 2016). Fumonisins contamination of stored maize in Tanzania was reported to be high with levels up to 11,048g/kg, which exceeds the maximum tolerable limit set by the East African Commission (EAS, 2:2011) which is (2000 µg/kg) (Kimanya *et al.,* 2008). The level of fumonisins exposure in children through maize based complementary foods exceeds the provisional maximum tolerable daily intake of 2µg/kg body weight. The exposed group of children were significantly shorter than other unexposed group, hence revealed association between fumonisins exposure and growth retardation (Kimanya *et al.*, 2010).

2.3 Effects of Fumonisins on Human Health

Fumonisins $B_{1,}$ B_2 and B_3 commonly found in maize and maize products, has a long chain of hydrocarbon with similar structure to sphingosine which plays a major role in their toxicity effects to disrupt the function of sphingolipid metabolism (Merrill *et al.*, 2001; Heidtmann-Bemvenuti *et al.*, 2011). Fumonisin B_1 disrupts ceramide synthase, the major enzyme in the sphingolipid biosynthesis pathway, thereby resulting in inhibition of the signalling pathways and cell functions of the sphingoid bases and complex sphingolipids, which is the cancer promoting metabolites in human (Marin *et al*., 2013).

The sphingolipids play important roles in membrane and lipoprotein structure, cell-to-cell communication, interaction between cells and extracellular matrix and regulation of growth factor receptors (Soriano *et al.*, 2005). Fumonisins have been reported to cause neural toxicity diseases in horses and cardio toxicity in pigs; while the effects of fumonisins in human are uncertain due to exposure dose and duration, although the epidemiological studies around the world have linked the incidence of human oesophageal cancer and exposure of fumonisins (Knudsen *et al.,* 2011; Anukul *et al.,* 2013). It has been reported that fumonisins exposure may become etiological agent of oesophageal carcinoma to human in Transkei region in south Africa and interfere with cellular folate uptake that cause neural tube defects in foetus (Anukul *et al.,* 2013). Exposure to fumonisins is also known to cause growth impairment to children, liver and oesophageal cancer (Marin *et al.,* 2013).

The study conducted to explore the influence of fumonisins exposure on the growth performance among children fed on maize based complementary foods contaminated with fumonisins showed that exposure to fumonisins reduce growth rate in height and weight (Kimanya *et al*., 2010). Exposure to fumonisins through cereals based complementary flour or other food stuffs was associated with child growth impairment, due to the fact that fumonisins in human body tend to disrupt sphingolipid metabolisms and disrupt intestinal permeability, hence interfere with nutrient absorption and lipid synthesis which affect growth pattern (Soriano *et al.*, 2005; Kimanya *et al*., 2010; Marin *et al.,* 2013).. Fumonisins contamination can also cause depletion of sphingolipids which in turn inhibit uptake of folate in different cells. This cellular deficiency is known as a cause of neural tube defects to children (Gheysen, 2015).

2.4 Cereal-legume Complementary Flour

Complementary foods are the introductory food given to children at six months of age in addition to breast milk (Fikiru *et al.,* 2016). In Tanzania complementary flour is composed of cereals, legumes, nuts and some seeds. The most common cereals used in home-based complementary flours are maize, sorghum, millet, rice and wheat, while the legume used are soy and beans (Muhimbula *et al*., 2011). These products are being used in other people in needy like sick people, pregnancy and lactating woman. It is identified to offer additional nutrients to the mentioned group, because during that period is the window of opportunity for malnutrition to under five and people with special needy like sick, lactating and pregnant woman (Compaoré *et al*., 2011). Complementary flour need to be nutritionally adequate and microbiologically safe, with regards to its intended use to the mentioned group (Maseta *et al.,* 2016). In developing country like Tanzania, would be difficult to avoid microbial contamination to the cereals used in making complementary flour due to lack of personal hygiene, lack of clean water and poor food handling during processing and handling causing microbial, chemical and physical contamination (Kulwa *et al*., 2015).

Complementary foods are non-human-milk food-based sources that are given to children when breast milk alone is no longer sufficient to meet the nutritional requirements. Appropriate complementary feeding should start from the age of six months with continued breastfeeding up to two years of age or beyond. . In many countries in Africa, complementary foods are based on cereal flour, boiled in water. The composite flours that are common in many communities have a cereal/legume ratio of 70:30 (Dewey and Adu-Afarwuah, 2008; Mbithi-Mwikya *et al*., 2002; WHO, 1998; Gheysens, 2015). Household technologies such as fermentation, soaking, roasting and malting are traditionally used in many African societies. These practices can contribute to improving the safety and quality

of complementary foods. However, reaching an adequate nutrient level remains a concern, particularly in diets that are mainly plant-based (Muhimbula *et al.,* 2010). In Tanzania, the main complementary food consumed by children is a thin porridge prepared from maize flour with varying ingredients and cooking methods (Kulwa *et al.,* 2015). The composition of complementary foods depends on the age of children. Children aged 3-5 months consume a very thin maize porridge while older children aged 6-11 months receive a much thicker maize or composite flour porridge. Other cereals used for complementary feeding are sorghum, wheat, rice and finger millet (Mamiro *et al.,* 2005; Gheysens, 2015).

The daily average energy requirements from complementary foods for children in developing countries is approximately 200 kcal at 6–8 months, 300 kcal at 9–11 months and 550 kcal at 12–23 months (Abeshu *et al*., 2016). These values represent 33 %, 45 % and 61 % of total energy needs respectively (WHO, 2002). The amount of protein needed from complementary foods increases from about 2 g/day at 6–8 months to 5–6 g/day at 12–23 months, with the percentage from complementary foods increasing from 21% to about 50% (WHO, 2003). In developing country like Tanzania, would be difficult to avoid microbial contamination to the cereals used in making complementary flour due to lack of personal hygiene, lack of clean water and poor food handling during processing and handling causing microbial, chemical and physical contamination (Kulwa *et al*., 2015).

Most millers pack flour in paper bags, polythene bags or paperboard cartons, Cardboard, polythene and paper packaging is widely available and can usually be printed by local print companies. However the post harvest operation such as sorting and grading, ingredient inspection, process control, cleaning schedules and control over packaging and

distribution and storage are still challenges. The basis of this is to ensure safety and quality product in relation to mycotoxin contamination especially fumonisins.

2.5 Impact of Type of Packaging Material on Fumonisins

Packaging on products is primarily used to protect and preserve packed flour; however it can also provide valuable marketing information to the producer (Meyers and Lubliner, 1998). Food product packaging can be a factor in consumer decision-making, because it enables consumers to make assumptions about how the product tastes. In some cases in addition to shaping taste expectations, the packaging can even affect subsequent product safety and quality of contents. In modern age, food packaging has become very important because of protection of the product from contamination by macro and micro-organisms and their filth, prevention from loss or gain of moisture, shielding the product from oxygen and to facilitate handling (Ball, 1960).The impact of packaging material on fumonisin contamination was not well established. In tropical climate like Tanzania the shelf life of flour can be a serious problem and due to weather conditions, post-harvest handling and storage practices have influence on production of fungi that produces toxins (Kamala *et al.,* 2016)..

2.6 Chemistry of Fumonisins

Fumonisins are a group of polyketide-derived mycotoxins that have a wide geographic distribution, and are consequently most commonly present on maize in many different regions (Marasas et al., 1996). Fumonisins can be separated into four main groups, identified as the fumonisin A, B, C, and P series (Bartók *et al.,* 2006); the B group includes the most active fumonisins FB_1 and its isomers FB_2, FB_3 and FB_4 (Bartók *et al.,* 2010). They are polar compound which is hygroscopic and soluble in methanol solution (Yu *et al.,* 2014). The structure of fumonisins suggests a biological interplay that could

explain the physiological effects of these mycotoxins. Although up to 13 Fusarium species are able to produce fumonisins (Marín *et al.*, 2004; Ferigo 2016), *F. verticillioides* and *F. proliferatum* are the most important species associated with fumonisin contamination. Fumonisin B_1 and FB_2 produced in crop when moisture content were high and are stable during storage, although are unstable at high temperature range above 120 ^{0}C (Yu *et al*., 2014)

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Dar-es-salaam region of Tanzania, although it involved the collected samples manufactured from different region of Tanzania that marketed in Dar-es-salaam. The region experiences the tropical climatic condition that accompanied with hot and humid weather condition through out of the year (NBS, 2014). It boarders Coast Region in the North, West and South while to the East there is the Indian Ocean.

3.2 Sample Collection

A cross-sectional design was used in data collection to determine the levels of fumonisins in cereal-legume-complementary flour. Cereal–legume complementary flour samples (n=60) were randomly collected from all municipalities (Kinondoni, Ilala, Temeke, Ubungo and Kigamboni) in Dar es Salaam region. For each sample one kilogram of cereal-legume complementary flour was purchased randomly from selected open markets vendors, shops and groceries. The samples were kept in their original packaging materials and taken to Tanzania Food and Drugs Authority (TFDA) laboratory in Dar es Salaam and stored at 19 – 20 ^{0}C before analysis of fumonisins and moisture content.

3.3 Sample Size Determination and Study Design

A cross-sectional study design was used to establish the levels of fumonisin contamination in cereal-legume complementary flour marketed in Dar es Salaam. The sample size determined using Kirkwood formula (2003).

$$N = \frac{[\ (p(1-p)½ + V(Pnull(1-Pnull)½]2}{(p-Pnull)2}$$

Whereby,

N = required minimum sample size

p = the proportion of interest

Pnull = the null hypothesis

μ = the one sided percentage point of the normal distribution corresponding with 90%

V = percentage of normal distribution corresponding to the 5% level of significance (1.96).

3.4 Quantification of Fumonisins

Fumonisins B1 and B2 in the cereal-legume complementary flour were determined by ISO 16050 using High-Performance Liquid Chromatography (HPLC) method as described by Sydenham *et al*. (1992) and the slight modifications made by Samapundo *et al.* (2006).

3.4.1 Standard curve preparation

A stock standard solution containing 50.3 ng/ml -fumonisin B_1 and 50.7 ng/ml fumonisin B_2 in acetonitrile: water (50:50 v/v) (RomerLabs, Diagnostic, Austria) was used in the analysis. A working standard solution containing 5000ng/ml each fumonisins B_1 and B_2 was prepared in acetronitile : water to make solution for the calibration curve. The curve was prepared between the concentrations ranging from of 45 ng/ml and 270 ng/ml. HPLC grade methanol and acetonitrile solution were supplied by Fisher Scientific (Leicestershire, U.K.).

3.4.2 Extraction of FB_1 and FB_2

The extraction procedures were done according to the method described by the ISO 16050 (2003). Each of the collected samples of cereal-legume complementary flour was

analysed independently to determine levels of fumonisins. For each sample, a weighed 15 g portion was weighed into a 100ml conical flask and mixed with 40 ml of methanol: water (3:1, v/v). The conical flask glass was fitted on a horizontal advanced orbital shaker (Tallboys' shaker, standard model 3500, (Langenselbold, Germany) and shaken at 200 rpm for one hour. The mixture of cereal-legume composite flour and the methanol water was filtered through folded filter paper (Whatman No.1) into Erlenmeyer flask for 10 min to remove the residue of flour so as to get clear solution. Ten mililitre of filtered mixture was mixed with 40 ml of Phosphate Buffered saline (PBS), and then mixed thoroughly for 5 min. The pH of filtrate mixture was adjusted between 5.8 and 6.5, using 0.1 M sodium hydroxide before the clean-up procedure.

3.4.3 Clean up for FB_1 and FB_2

The 10-ml aliquot of the filtered extract was applied to a strong anion exchange (SAX) cartridge (Varian, Bond-Elut LRC, 500 mg, Varian Belgium NV/SA, Sint-Katelyne-Waver, Belgium) fitted to a solid-phase extraction manifold (Alltech, 24-Port SPE Vacuum Manifold System, Alltech Associates Inc., Lokeren, Belgium). After application of the extract, the SAX cartridge was then washed with 10 ml of the methanol–water (3:1, v/v), followed by 3 ml of pure methanol. The fumonisins were eluted from the cartridge with 10 ml of 98% (v/v) glacial acetic acid in methanol. The eluate was collected and evaporated to dryness at 60^{o}C under a gentle stream of nitrogen using a nitrogen evaporator (Pierce model 18780, Reacti-Vap) coupled with a dry bath (Pierce Reacti-Therm), both supplied by Rockford (IL, USA).

3.4.4 Preparation of the mobile phase

Mobile phase was prepared for use in HPLC which contained 750 ml of methanol and 250 ml of 0.1M NaH_2PO_4 (BDH) with pH adjusted to 3.35 using phosphoric acid

solution, then filtered under vacuum chamber using micron filter paper (0.45mm) before the use. The flow rate of the mobile phase to the HPLC system was set at the pressure of 1ml/min.

3.4.5 The HPLC system

The HPLC system contained stainless steel column, waters Spherisorb® 5um ODS 14.5 ×200mm (Milford, MA, USA). The machine used to pump twenty microliters of solution from 200µl a mixture of acetonitrile: water (1:1) and 200 µl of derivatising reagent which were added into dried fumonisins in the test tube and vortexes for at least 1 min, then injected into the HPLC system (Shimadzu, Tokyo, Japan). The detection of fumonisins in this system were done using florescence detector that were set at wavelength of 335 nm excitation at 440 nm emission. The limit of detection of the analytical method was 53 µg/kg for fumonisin B_1 and 47 µg/kg for fumonisin B_2.

The derivatizing reagent was prepared by dissolving 40 mg of ortho-pthaldehyde (OPA, Sigma-Aldrich, St. Louis, MO, USA) in 1 ml of methanol, 5 ml of 0.1M sodium tetraborate (BDH, Poole, UK) and 50 µl of ß-mercaptoethanol (Fluca, Steinheim, Germany). The HPLC system was equipped with a pump LC 20AD and fluorescence detector model RF-10AXL (Shimadzu, Tokyo, Japan), which were set at wavelength of 335 nm excitation and 440 nm emission. The HPLC system oven temperature ranged from 19ºC to 25ºC.

3.5 Determination of Moisture Content

Moisture content of the flour samples was determined according to AOAC (1999) using air oven method (1) at 105 ^{0}C for 3 hours.

3.6 Estimation of Children Exposure to Fumonisins and Risk Characterization

Estimation of children to fumonisin through contaminated cereal-legume complementary flour was done by considering mean daily intake of composite flour per day, multiplied by the mean average of total fumonisins contamination determined in the cereal-legume complementary flour; divided by child body weight in (kg) as per procedure described by Kimanya *et al.* (2008). The amount of complementary flour assumed was 40-90 g was offered at six to eight months, 200-280 g at nine to 11 months, and 380-500 g at 12- 23 months, of age in a day (WHO, 2001). The body weight of children determined from reproductive health card (RCH).

The risk was characterized in term of exposure above or below the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight per day as recommended by WHO (2012). Children with mean and median of exposure levels above PMTDI of 2 µg/kg body weight per day were categorized as high exposure group, while those with levels below PMTDI were categorized as the low exposure group (WHO, 2012).

3.7 Statistical Analysis

The data were analysed using R Software (R® version 3.3.1, Stanford University, USA). The linear regression model was used to determine relationship between level of fumonisins contamination and other variables among samples. Dependent variable was levels of fumonisin concentrations while the independent variables were product type, municipality and type of packaging materials. The statistical comparisons of data for levels of fumonisin concentration between regions were performed by one-way analysis of variance (ANOVA).The mean separation to establish differences in levels of fumonisins amongst packaging materials were done using Tukey's test.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1.1 The Samples of Cereal-Legume Complementary Flour

The study samples of cereal-legume complementary flour (n=60) were collected from five municipalities in Dar es Salaam region. They were produced by 47 different manufacturers in five regions according to the labelling information. The flour samples were manufactured in Arusha (n=10), Mbeya (n = 4), Morogoro (n= 6), Coast (n= 4) and Dar-es-Salaam (n= 36) regions (Table 1). The collected samples of cereal-legume complementary flour were composed of maize, rice, sorghum, groundnuts and soy beans. Eighty six percent of the collected sample had a mixture of the cereals and legume with few percent which contains maize and soy beans, cashew nuts and sesame seeds. The proportions of maize in the composite flour were high than the other ingredients based on the composition information from label.

Table 1: Distribution of sample by type of packaging materials and shelf life

Parameter				
	Aluminium	Box	Nylon	Paper
Number of samples	n (%)	n (%)	n (%)	n (%)
Packaging	2.0	13.0%	60.0	25.0
Shelf life of Products (month)	≤3	4 - 6	7 - 9	10 – 12
	13.0	45.0	28.0	13.0

4.1.2 Fumonisins contamination in cereal-legume complementary flour

The 100% of collected samples of cereal-legume complementary flour were contaminated with fumonisins at different levels (Tables 2a-c). The contamination of fumonisins B_1 detected in all flour samples produced from the region of manufacturers at the levels ranged from 2.94 – 60 669 µg/kg. The mean level of contamination was above acceptable

maximum limit for samples from Dar es Salaam (6 434.9µg/kg) and Arusha (2 095.2µg/kg) while the mean level for samples from Mbeya (127.3 µg/kg), Coast (184.4µg/kg) and Morogoro (214.4µg/kg) was within the acceptable limits (Table 2). The acceptable maximum limit of fumonisin contamination in a complementary flour sample is 2000µg/kg based on Tanzania standards for 2010 regarding cereals intended for human consumption.

The contamination of fumonisins B_2 in cereal legume - complementary flour ranged from 5.74 – 15758.8µg/kg, with mean level of 1303.31µg/kg, 622.35µg/kg, 82.32µg/kg, 63.04µg/kg and 58.19µg/kg for samples from Dar es Salaam, Arusha, Coast, Mbeya and Morogoro region respectively. The levels of fumonisin B_2 contamination was lower in all region compared to fumonisin B_1 contamination from the samples (Table 3).

Thirty five percent of the analyzed samples of cereal - legume complementary flour were contaminated with total fumonisins at levels above the acceptable limit of 2 ppm set out under East African Community standards for maize grains (EAS:2011). The prevalence of fumonisins B_1 and B_2 contamination in flour sample was higher in all regions. The mean levels of contamination were 7 057.3µg/kg, 3 398µg/kg, 272.3µg/kg, 266.8µg/kg and 190µg/kg in samples manufactured in Dar es Salaam, Arusha, Morogoro, Coast and Mbeya regions respectively (Table 4).

The results obtained from the present study showed that the mean levels of fumonisin B_1 contamination were lower than 6125µg/kg reported by Kimanya *et al.* (2008), 5461µg/kg reported by Gheysen (2015) in regarding maize based complementary flour; although the levels of contamination 1702µg/kg reported by Maseta *et al.* (2016) was lower than results determined from this study. The results from this study compare well with

4000-8000 µg/kg reported in Zimbabwe by (Gamanya and Sibanda, 2001). Sydenham *et al.* (1990); Shephard *et al*. (2007) reported similar fumonisin levels in the area of the former Transkei region of South Africa (maximum levels of 7900 µg/kg. A similar maximum level of 12 000µg/kg was reported in Benin by Fandohan *et al.* (2006).Doko *et al.* (1996; Kimanya *et al.,* 2008a) reported total fumonisin levels of 370 µg/kg for maize from Botswana, 4135µg/kg for maize from Malawi and 2735 µg/kg for maize from Zimbabwe; Relatively lower maximum level of 4222 µg/kg for total fumonisins was also reported by Kpodo *et al*. (2000) for maize in Ghana.

The detected concentration of 3273 µg/kg for total fumonisins was higher than 1 685.15 µg/kg reported by Maseta *et al.* (2016) and 1 745 µg/kg reported by Gheysen (2015) but lower than11048 µg/kg reported by Kimanya *et al.* (2008). The mean concentration for fumonisins B_1 and total fumonisin were higher compared to fumonisins B_2 in all regions of production regardless of the manufactures. The FB_1 was known to be the most prevalent and potent than the other fumonisins and has been categorized as probable carcinogenic in human which have toxicological significance in other animals (Wild *et al*., 20104). The exposure to fumonisins at any levels is recognized to affect child health since it has cumulative effects (Kimanya *et al.*, 2010).

The levels of fumonisins contamination observed in this study could be attributed to the climatic characteristics of areas where samples were grown. Most of the regions have been reported to have high incidence of mycotoxins contamination especially fumonisins and aflatoxins (PACA, 2016). The regions represented three Agro Ecological Zone with various climatic characteristics. Arusha region in the Northern highlands, Coast, Dar es Salaam and Morogoro regions in the Eastern lowlands and Mbeya region in the Southern highlands. These zones experiences tropical and subtropical type of warm climate with

temperature range 20-25 ^{0}C, varying during the day and humid climate throughout a year that affect growth of Fusarium species in cereal grains (Czembor *et al*., 2015). According to Michael and Wyatt (1993); Mwalwayo and Thole, (2016), Grains grown in tropical and subtropical regions are more prone to mycotoxins contamination particularly fumonisin due to the relatively long and warm growing season.

According to Devide *et al*. (2016) fumonisins concentration can exceed the maximum and tolerable limits under different environments in particular when the weather conditions are not stable in addition to inadequate management strategies during pre and post harvest practices. It is considered that humidity, draught stress, temperature, and rainfall are among the important factors that can affect contamination of agricultural products, example the higher fumonisins contamination reported in maize fields in Uganda was attributed to higher humidity and inadequate agronomic practices (Santiago, Cao and Butrón, 2015).

The high level of contamination by fumonisin observed in Dar es Salaam region and Arusha region could be associated with climatic environment of the areas. For example Dar es Salaam region is characterized by high temperature and humidity which is conducive environment for cereal infection by Fusarium fungi. Popovski and Celar, (2013) reported that temperature and humidity (wetness) are the main climatic factors influencing the development of *Fusarium* fungi in store. Since the major cause of fumonisin contamination in composite flour is the presence of maize. High proportional of maize content in the formulation of flour samples then might contribute the increased fumonisin levels and vice versa.

Table 2: Concentration of fumonisin B_1 (ng/g) in cereal-legume complementary flour marketed in Dar-es-Salaam

Location/Region of manufacturer	Number of samples analyzed	Concentration of fumonisin B_1 (ng/g)	Number of samples with concentration above maximum limit (*2000 µ*g/kg)	Range of contamination (Min-Max) (*n*g/g)	Mean contamination (*n*g/g)
Arusha	10	[25.69, 54.61, 103.85, 188.90, 48.70, 344.07, 2599.42, 303.43, 11.38]	2	11.4-2599.4	2095.2
Coast	4	[71.37, 125.68, 475.90, 64.80]	0	64.8–475.9	82.3
Dar-es-Salaam	36	3312.52, 1190.95, 341.40, 1144.70, 553.22, 438.80, 54.25, 33.35,94.51, 89.45, 7728.00, 176.95, 2739.41, 39568.54, 479.64, 1428.00, 4.17, 463.43, 4.54, 4.23, 9.25, 24.64, 55.45, 56.63, 993.25, 2793.25, 140.66, 71.14, 29.53, 9345.48, 1060.42, 51.71, 37.48, 64.38, 276.60, 567.04]	9	4.2–39568.3	6434.9
Mbeya	4	[382.60, 25.26, 42.65, 58.53]	0	25.3–382.6	127.3
Morogoro	6	[2.94, 42.46, 64.97, 85.80, 1082.38, 6.23]	1	2.9–1082.4	214.1

Table 3: Concentration of fumonisin B_2 (ng/g) in cereal-legume complementary flour marketed in Dar-es-Salaam

Location/Region of manufacturer	Number of samples analyzed	Concentration of fumonisin B_2 (ng/g)	Number of samples with concentration above maximum limit (*2000* *μ*g/kg)	Range of contamination (Min-Max) (*n*g/g)	Mean contamination (*n*g/g)
Arusha	10	[27.53, 28.65, 60.18, 55.68, 44.47, 817.80, 22.0, 1573.38, 3576.35, 17.46]	2	17.5–3576.4.0	622.3
Coast	4	[201.50, 65.74, 34.16, 27.87]	0	27.9–201.5	82.3
Dar-es-Salaam	36	[1339.88, 1819.30, 1809.0, 15758.06, 108.00, 92.95, 54.25, 285.32, 54.76, 119.36, 1517.26, 15.20, 121.66, 380.75, 136.66, 91.83, 178.61, 64.19, 26.92, 178.61, 64.19, 26.92, 2429.44, 18.37, 6401.71, 41.40, 23.57, 1120.61, 834.94, 391.66, 90.19, 37.07, 7116.96, 28.68, 39.50, 15.53, 53.98, 1698.55]	9	15.2–15758.8	1303.3
Mbeya	4	[42.81, 9.30, 160.81, 39.23]	0	9.3–160.8	63.03
Morogoro	6	[27.13, 10.49, 58.17, 5.74, 201.87, 45.76]	0	5.7–201.9	58.2

Table 4: Concentration of Total fumonisin (FB_1+FB_2) (ng/g) in cereal-legume complementary flour marketed in Dar-es-Salaam

Location/Region of manufacturer	Number of samples analyzed	Concentration of total fumonisin (B_1 +B_2) (ng/g)	Number of samples with concentration above maximum limit (*2000* μg/kg)	Range of contamination (Min-Max) (*n*g/g)	Mean contamination (*n*g/g)
Arusha	10	[53.22, 83.26, 164.03, 244.58, 93.17, 39949.30, 366.05, 4172.8, 3879.79, 28.84]	2	28.8–39949.3	3398.5
Coast	4	[272.86, 191.42, 510.06, 92.67]	0	92.67–510.06	266.8
Dar-es-Salaam	36	[4652.40, 3010.25, 2150.40, 16902.75, 661.18, 531.75, 108.49, 318.67, 149.27, 208.82, 9245.26, 192.15, 2861.07, 61486.80, 616.30, 1519.83, 182.78, 527.62, 31.46, 2433.67, 27.62, 6426.35, 96.85, 80.20, 2113.87, 3628.19, 532.32, 161.32, 66.59, 16462.44, 3663.43, 80.39, 76.95, 79.91, 330.58, 2265.59]	15	27.6–61486.8	7057.3
Mbeya	4	[425.41, 34.56, 203.46, 97.76]	0	34.6–425.41	190.3
Morogoro	6	[30.07, 52.95, 123.14, 91.54, 1284.25, 52.0]	1	30.07–1284.25	272.32

4.1.3 Fumonisins contaminations in cereal-legume complementary flour by regions

The contamination of fumonisins B_1 (Figure 1) were higher above the acceptable maximum limit (2000 µg/kg) based on (EAS 2:2011) for the samples from Dar es Salaam and Arusha region as compared to the samples manufactured in Mbeya, Coast, and Morogoro regions which were within the acceptable limits. The levels of fumonisins B_2 contamination in these regions Arusha, Coast, Mbeya and Morogoro were within the acceptable limits with exceptional of Dar-es-Salaam which had contamination above the acceptable limits. Magoha *et al.* (2014) reported that 68% of the flour samples collected from Northern Tanzania was contaminated with total fumonisins concentration above the maximum tolerable limit (MTL). The samples of cereal-legume complementary flour from Dar es Salaam had higher levels of fumonisins concentration than the other region of production may be contributed to the sources of raw materials and the post harvest practices of raw materials like maize. Also the proportional of samples from Dar es Salaam were many than other region of sample production could mask the effect and shown to be high than the others. The lower levels of fumonisins concentrations from the samples produced in Coast, Morogoro and Mbeya region were contributed to the number of samples, although the other factor like types of flour, storage condition and type of packaging had less contribution on the fumonisin contamination.

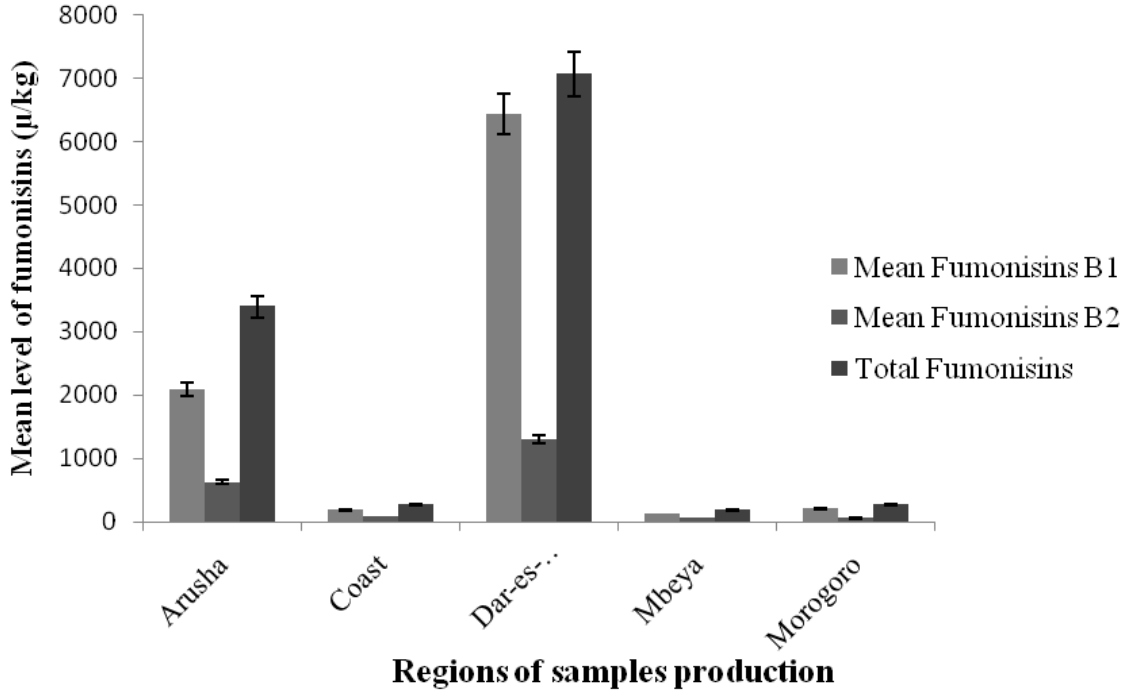

Figure 1: Mean levels of fumonisin concentration (µg/kg) in cereal-legume complementary flour by regions of production.

4.1.4 The effects of packaging materials on fumonisin contamination in cereal legume complementary flour

The commonly used packaging materials for cereal-legume complementary flour observed in the study were nylon packs, paper packs, boxboard packs and the aluminium pack. Most of the packaging were shielded by double pack in which the inner packs contained thin nylon while outside cover varied from paper packs to aluminium pack. The hard nylon (polyethene pack) and aluminium had a single pack while the thin nylon and other package were coved with double pack in which the thin nylon were common inside samples (Plate 1).

The level of fumonisins FB_1 and FB_2 contamination varied with types of packaging materials. The great variations were noted for hard paper and nylon covering materials. Table 3 shows the effects of packaging on total fumonisins with respect to FB_1 and FB_2.

The box board pack and nylon showed high levels compared to others type of package from the same products. The levels of fumonisin concentration on the hard nylon and boxboard package were (3061.6 µg/kg) and (1290.7 µg/kg) respectively. The level of fumonisins concentrations observed could be affected by number of sample of the cereal-legume complementary flour and composition of the flour. Although the fumonisin contamination observed to occur from field and less affected with post-harvesting practices as described by (Shaaban *et al*., 2015). other studies explained the effect of packaging material on quality of composite flour and aflatoxins concentration, rather than fumonisin occurrence due to the fact that fumonisins is known as the field mycotoxins (Mutegi *et al.,* 2013: Kamala *et al.,* 2015). The fumonisins contamination observed in the aluminium package was not significance due to the fact that the sample was single and therefore it was statistically incomparable.

Table 5: The effects of packaging on levels of fumonisins in cereal-legume complementary flour

Packaging material	FumonisinB_1 (µg/kg) Mean ± SE	FumonisinB_2 (µg/kg) Mean ± SE	Total Fumonisin (µg/kg) Mean ± SE
Box board	178.2 ± 151.2	75.6 ± 30.5	253.8 ± 181.6
Hard Paper	1290.7 ± 704.2	813.9 ± 452.7	2104.7 ± 1051.9
Nylon	3061.6 ± 1627.2	1087.7 ± 412.3	4149.3 ± 1687.5
Paper	61.2 ± 22.8	375.9 ± 342.3	437.0 ± 333.3

*Means values across columns indicate significant difference in fumonisins levels ($P \leq 0.05$) according to Tukey's HSD multiple ranks test.

The packaging process and packaging material could affect fumonisins concentration in the samples. During processing, milling and packaging of cereal-legume complementary flour, the affected raw material with fungal responsible for fumonisins can bind to various components used in making complementary flour causing contamination that

differentially distributes fumonisins in the resulting products (John and Antonio, 2014). Packaging played a very important aspects since it provide protection of the product from contamination by macro and microorganisms. They prevent product from loss or gain of moisture, shielding the product from oxygen and to facilitate handling as well as waste (Ball, 1960). Similarly good packaging extends the shelf life of the food by better protection from all the hazards during storage. Some packaging material tend to absorb moisture from the environment, while the other material like plastic pack retain heat and moisture in the bags which promote fungal growth and mycotoxins contamination (Mutegi *et al.*, 2013). From the study, the suitable packaging material in protection of fumonisin in cereal-legume complementary flour is a nylon which covered by paper package outside.

4.1.5 The mean moisture content in packaged cereal-legume complementary flour

The average moisture content of the collected cereal-legume complementary flour varied from 8.5 to 14.4%, the mean level of 11 % (v/v) for cereal-legume complementary flour samples. The seventy five percent of mean moisture content of the cereal – legume complementary flour was 10% (v/v) of all sample collected and 25% were above 13% (v/v) (Figure 2).

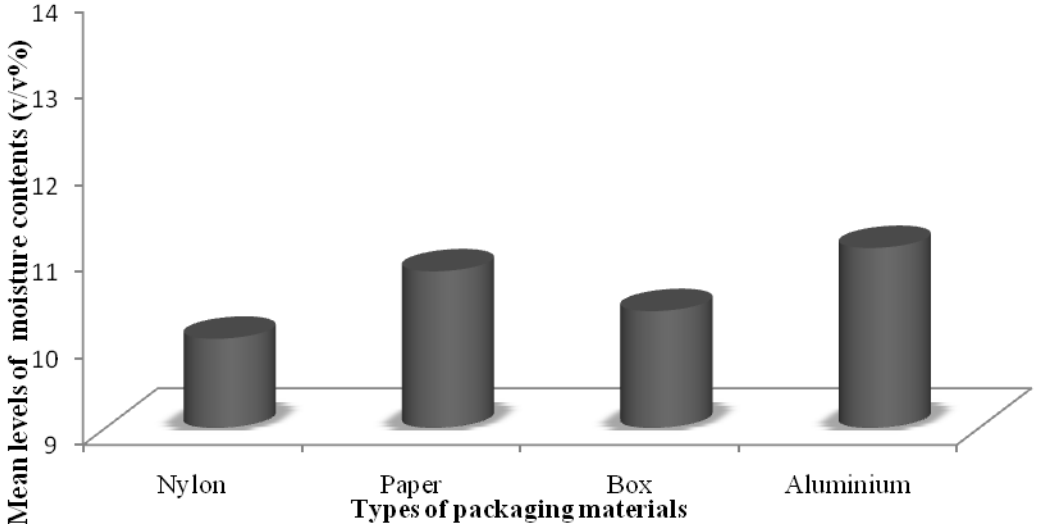

Figure 2: Moisture contents of packaged cereal-legume complementary flour.

Moisture content of flour is essential regarding to its shelf life, nutritional quality and microbial growth; it has significant effect on crude protein, crude fat higher in cereal-legume complementary flour having moisture content above 13 % (v/v) (Mutegi *et al.*, 2013). The lower moisture content in cereal -legume complementary flour is the better for its storage stability, less microbial contamination and nutritive quality (Nassir *et al.*, 2003). The Moisture content below 13% (v/v) has less effect on fumonisin contamination during storage while the fumonisin contamination occurs in the field when the grain had moisture content above 13% (v/v) and high relative humidity (Temba *et al.*, 2016). The recommended moisture contents for flour are 13% (v/v) AOAC (1999). Therefore the mean moisture obtained in the study was within the recommended range for proper storage of the cereal legume complementary flour for most samples. The moisture content observed from the cereal-legume complementary were with the acceptable range of 135(v/v) and showed negative relationship with fumonisin concentration.

4.1.6 Assessment of level exposure to fumonisins from contaminated cereal-legume complementary flour in children underfive years of age

Assessment of exposure of children to fumonisins from contaminated cereal legume complementary flour was based on total fumonisins (FB_1+FB_2) and amount of flour consumed per day and body weight. The study results indicated that children are exposed to fumonisins at levels ranging between 0.1 to 14.5 (µg/kg body weight/day) for infant aged 6 – 8 months and 0.1 to 13.4 (µg/kg body weight/day) for children aged from 9 to 23 months. The mean and median level of exposure to total fumonisins for children aged 6 - 8, 9 – 11 and 12 - 23 months were (14.5:1.1µg/kg body weight/day), (13.6:1.0µg/kg body weight/day) and (13.6:1.0µg/kg body weight/day) respectively (Figure 3).

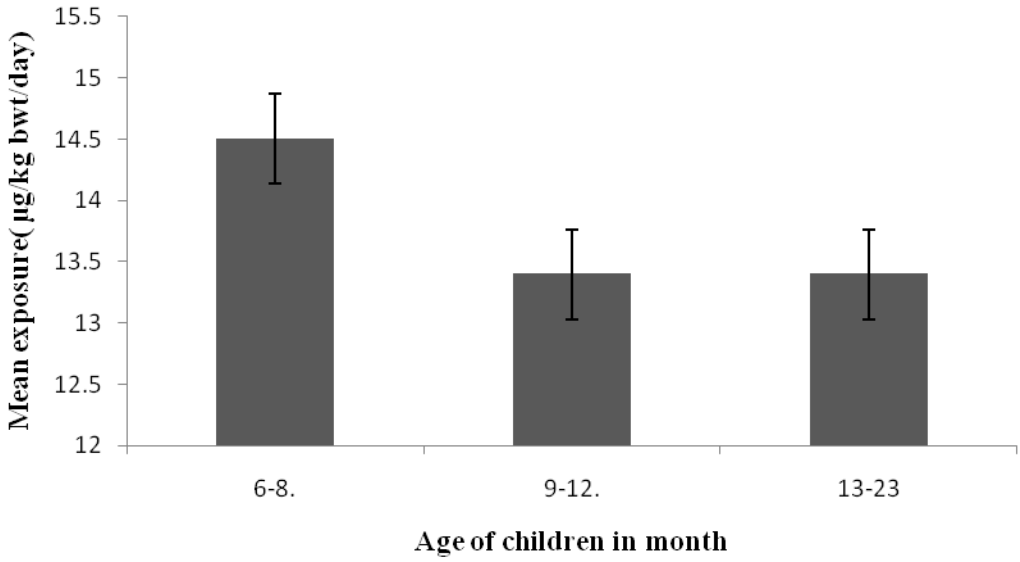

Figure 3: Exposure to fumonisins in children underfive years of age

4.1.7 Risk categorization

The risk categorization depends on the level of fumonisin exposure from contaminated cereal-legume complementary flour. Generally the risk of exposure to fumonisins through cereal-legume complementary flour under this study was (22%) who were exposed above 2µg/kg bwt/day and 78% were exposed to lower than 2µg/kg bwt/day, the fumonisins

contamination at any levels could have health hazards due to chronic effects*;* This means food which was given to children and other needy groups in the community were less safe and exposed individual to fumonisins at different levels that may contribute to the health effects associated with fumonisins contamination.

The children were exposed to fumonisins at levels that exceeded 2µg/kg by body weight per day for the baby foods (EAC, 2011). Magoha *et al*. (2014) reported that 29% of the infants under six months of age were exposed to FB_1 through breast milk and the exposure of FB_1 was above the provisional maximum tolerable limit of 2µg/kg body weight per day. Kimanya *et al.* (2010) reported that 26% of children were exposed to fumonisins above the maximum provisional tolerable limits through maize-based complementary foods in Northern Tanzania. The study results on risk categorization for fumonisin exposure to children were lower compared to reported results; this could due to effect of cereal mixing of rice, sorghum wheat and soy which are less contaminated by fumonisins. Also the rate of consuming cereal-legume complementary flour was decreasing with age, which is the reason for the children from six to nine month were much affected than the other group.

The early exposure of fumonisins during child hood is detrimental to their health as it affects central nerve system leading to neural tube defects (NTDs) (Wild and Gong, 2010), Cardiovascular refs Gastrointestinal and Immune system depending on the exposure rate and the stage of the growth (Smith *et al*., 2012; Kimanya *et al*., 2010). Although fumonisins does not directly damage DNA, it contains components that induces cancer (IARC, 2002) and is a cancer promoter (Carlson *et al.*, 2001; Gelderblom *et al.*, 2002). The exposure to fumonisins through complementary food to infant has shown the linear association with impaired growth to the exposed infant than non-exposed (Kimanya

et al., 2010; Magoha *et al*., 2016). The high incidence of esophageal cancer was associated with high consumption of food contaminated with fumonisins above the tolerable limits in many studies in Africa and Europe (Wild *et al*., 2015). It is reported that the exposure to fumonisins leads to neural tube defect to children's in line with immunity suppression which may lead to stunting due to episodes of diarrhoea (Smith *et al*., 2012; Wild and Gong, 2010).

Feeding practices that were sorely based on cereal-legume based complementary flour/foods exposes children who are under-five years of age to risks of fumonisins contamination. It is believed that cereal based complementary flour has nutritional significance for preventing children from malnutrition (underweight, wasting and stunting) (Kulwa *et al*., 2015). Although is given as the additional food to supply nutrients such as carbohydrate, energy, proteins and micronutrients in addition to breast milk after six month, when breast milk is insufficient to fulfil child need at 6 month of age and above (Muhimbula *et al*., 2011; Pitt *et al.*, 2012).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results revealed fumonisins contamination in cereal-legume complementary flour given in addition to breast milk to under five children after six month of age. Similarly 22% of children are likely to be exposed to fumonisin through cereal-legume complementary flour that used as complementary food above the Provisional tolerable daily intake (PMTDI) recommended by World Health Organization. Packaging materials were observed to have contribution on build up of fumonisins in the product. However this may be due to inherent moisture during milling of grains and packing of commercial cereal flour product. Improper post harvest practices such as sorting of affected grains, dehulling of processed cereal-legume composite flour may be predisposing factor for fumonisins growth and multiplication on the grain and consequently to the finished flour.

5.2 Recommendations

Fumonisins contamination in most commonly used cereal-legume complementary flour may expose consumers to health effects associated with the toxins. Efforts are needed to intervene the situation in order to have safe food for children and general population. Interventions should focus on reducing mycotoxins by using simple and cost-effective ways to the community; this could be achieved by creating awareness on the effects of mycotoxins to health and measures to address the problem. As a matter of facts the control cannot rely on finished product itself rather the strategies should integrate the following:

i. Proper sorting of grains intended for preparation of cereal-legume complementary flour, this should involve separation of damaged grains, separation of clean grains from those having molds colonization.

ii. Use of proper packaging material especially double covering, inside cover with aluminium. This will help to minimize chance of moisture penetration and insect infestation in the product.

iii. The area used for packing of flour should be clean since it could be a good source of inoculums for mycotoxin especially Fusarium spores.

iv. The packed flour should be stored on shelf with relatively low temperature since high temperature can enhance fungi multiplication and hence mycotoxins accumulation.

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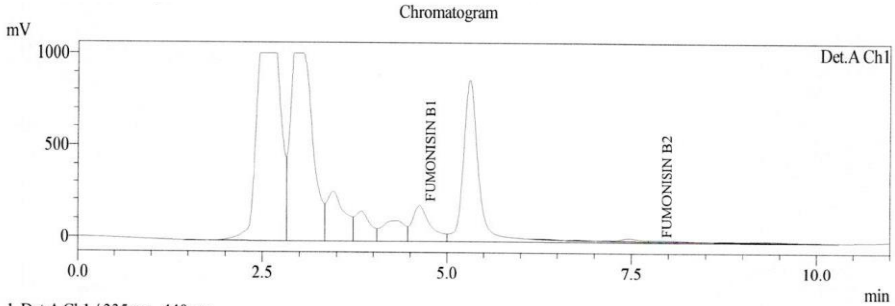
APPENDICES

Appendix 1: Chromatography results for analyzed sample



1 Det.A Ch1 / 335nm - 440nm

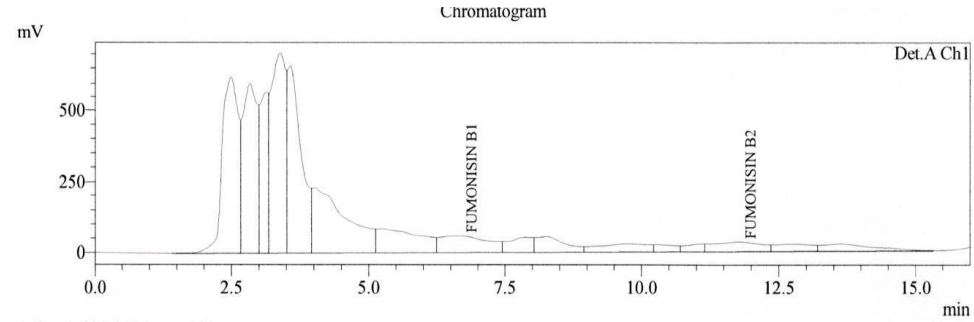
<Results>

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	FUMONISIN B1	4.621	3363575	198473	58.532	ng/g	V
2	FUMONISIN B2	7.823	137725	4948	-39.225	ng/g	TV

Calibration Curve



1 Det.A Ch1 / 335nm - 440nm

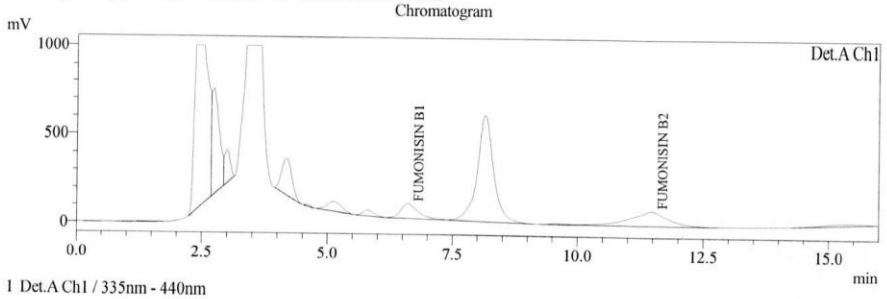
<Results>

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	FUMONISIN B1	6.661	3531419	57134	64.966	ng/g	V
2	FUMONISIN B2	11.766	2001467	32803	58.171	ng/g	V

Calibration Curve



1 Det.A Ch1 / 335nm - 440nm

<Results>

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	FUMONISIN B1	6.595	1945384	80506	4.171	ng/g	
2	FUMONISIN B2	11.468	4306202	81471	178.611	ng/g	V

Calibration Curve