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COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

ASSESSING FUMONISIN B1 LEVEL IN MAIZE AND MAIZE PRODUCTS FROM THREE MAJOR MARKET CENTRES IN KUMASI

BY

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A Thesis submitted to the Department of Food Science and Technology, College of Science

in partial fulfilment of the requirements for the degree of

MSc. Food Science and Technology

May, 2018

DECLARATION

I hereby declare that this submission is my own work towards the MSc. and that, to the best of my knowledge, it contains no material previously published by another person, nor

material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ACKNOWLEDGEMENT

I wish to express my sincere thanks to the Almighty God for how far He has brought me in life, for His unfailing love and undeserved kindness.

My biggest gratitude to my supervisor Dr. Francis Alemawor for his support and supervision which encouraged me to bring out my best to produce this work. My sincere thanks to my Head of Department, and all other lecturers in the Department of Food Science and Technology and friends for supporting me in diverse ways throughout my programme. A very big thank you to my parents, Mr. Robert K. Kesse and Georgina Gyamfuaah and the entire family for their care, support and love.

I also thank Mr. William Ofori-Appaw for granting me the permission to use the Mycotoxin Laboratory and to all staff members for their support throughout my work.

A special thanks to Damian Laryea, Redeemer and Nana Kwame Osei Agyeman whom out of their busy schedule have been very supportive throughout the entire work.

May the good Lord continue to bless and strengthen you all as you continue your good works.

ABSTRACT

Maize is one of the staple cereal crops consumed in Ghana together with its products such as maize-flour and maize-dough. These are mostly contaminated with mycotoxins such as aflatoxins and fumonisins. Fumonisins B_1 pose a major health risk for humans that consume large amounts of maize-based products and also domesticated animals. The aim of the study was to establish the incidence of fumonisin in maize and maize products. A total of 63 samples of maize, maize-dough and maize-flour of 1 kg each were randomly purchased from Asafo, Kumasi Central and Bantama markets in the Kumasi Metropolis. Percentage incidence of FB_1 level in both the maize-dough and maize-flour were 100% and maize was 81.82%. In Asafo market maize-dough recorded 24.08 mg/kg which exceeds the recommended limit followed by maize-flour 9.89 mg/kg and maize 0.09 mg/kg. In Bantama, maize-flour also had high level of FB_1 12.07 mg/kg followed by maize-dough and maize. Central market also recorded highest

level of FB_1 in maize-flour 9.67 mg/kg, maize 0.06 mg/kg and dough 2.58 mg/kg. From the results obtained maize-flour had FB_1 which exceeded the recommended from all the three markets. There were significant differences ($p<0.05$) between the FB_1 levels in maize (0.62) and the other maize products. The levels of FB_1 in the maize flour and dough confirm that consumers are at high risk of exposure to diseases associated with the fumonisins.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background Study

In Ghana, maize is one of the most important cereals crops produced and also the most widely consumed and is considered as staple of the people with increasing production since 1965 (Morris *et al*.,1999; FAO, 2008). Maize is mostly cultivated in the agro-ecological areas in Ghana such as the Upper East, Southern regions, Upper West and the Northern region. With about 1,000,000 hectares' accounts for the area planted and a total of 50%-60% for production of cereals (MoFA, 2013) and annual maize production is 1.5 million metric tons (Rondon and Ashitey, 2011).

Maize is a rich source of carbohydrates, protein, iron, vitamin B and mineral and provides 50% of the basic calories in humans' diet. These nutrients found in maize also helps prevent hemorrhoids, reduces cholesterol absorption in the body, controls diabetes and hypertension, soothes skin rashes and irritations, prevents various cardiovascular diseases, lowers risk of colon cancer, increases bone strength, boosts immune system and prevents anemia. It is also used in the production of animal feed such as poultry and livestock.

Maize has a high economic value since every part of its plant (the leaves, grains, stalks, tassel and cobs) is used to produce variety of foods (maize-dough, maize-flour, grits, beer, pastes), and non-food products (decoration of fabrics, curtains, jewelry and building materials). Though, maize serves as a rich source of energy and nutrients and has high economic value, it also has a high risk to be contaminated with mycotoxins such as aflatoxins and fumonisins (Doko *et al*., 1996; Miller, 2008). From the Food and Agricultural Organization of the United Nations, mycotoxin contaminate 25% of the continent's food crops (WHO,2002). Health of humans and livestock produce and trade are being affected greatly by these toxins globally (Wagacha & Muthomi, 2008; Darwish *et a*l., 2014).

Fumonisins pose a major health risk for high consumption by humans of maize and maize products and also domesticated animals. In humans, health problems such as oesophageal cancer, growth retardation in children, cardiovascular effects and neural tube defects in developing foetus are caused by fumonisins. Pigs also develop pulmonary edema when fed with feeds contaminated with the toxin (Kimanya *et al*., 2012; Marin *et al*., 2013). The consumption of these toxins are common in many rural African towns that ingest 500g of cereals daily as their main diet (Shephard *et al*., 2007), it affects organs such as the brain, lungs, liver, kidney (European Commission, 2000).

These toxins have been isolated and characterized to be more than 100 types with the major ones being fumonisins B_1, B_2 and B_3. Out of the three produced fumonisins, fumonisin B_1 is the most dominant and very lethal (Musser & Plattner, 1997) in human diet. Diarrhea and abdominal pain that was an outbreak in India was found to be as a result of the consumption of moldy maize contaminated with fumonisin B_1 (Bhat *et al.,* 1997). Therefore, ignorance of the public about the existence of these toxins has led to poor regulatory mechanism and dumping of food products. Many surveys have been conducted on the isolation and characterization of both the species and their toxins, example is the fumonisins in Maize hi and their co-occurrence with aflatoxin (Kpodo *et al*., 2000). Temperature, humidity, rainfall, drought stress during pre-harvest and post-harvest of maize accounts for the influence of fumonisins levels.

1.2 Problem Statement

Mycotoxins are toxins or secondary metabolites produced by molds during the stationary phase of their growth cycle. The environmental factor at the time of harvest and during storage mostly leads to the development of molds and subsequent formation of the mycotoxins (Bhat *et al*., 2000). Most molds associated with food and feed spoilage belong to genera *Aspergillus, Penicillium, Fusarium, Mucar* and others (EL-Shanawany *et al*., 2004). These toxins are

consumed by humans which is often undetected due to lack of public awareness concerning its presence in food and toxicity, lack of proper regulatory mechanisms and also lack of regulatory agencies to educate people about mycotoxin toxicity associated with consuming and handling contaminated foods. However, during both cultivation and storage of maize, specifically molds mainly *Fusarium* species such as *Fusarium verticilloides*, *Fusarium proliferatum* and several others grow on these commodities and produce the toxin known as the fumonisins and are harmful to both humans and animals (Frederiksen and Odvody, 2000).

1.3 Justification

Due to its occurrence in maize, the regulatory limit for direct human consumption in the United States is from 2 to 4 mg/kg (4 ppm), (FDA, 2001). For both direct human consumption and further processing, in European Union is 1 mg/kg (1ppm) and 4 mg/kg (4 ppm) respectively. The tolerable daily intake limit of fumonisins B_1, B_2 and B_3 alone or combined maximum as set by the FAO /WHO is 2 µg/kg body weight per day. The suggested regulatory limit for Fumonisin B_1 and B_2 by Codex Alimentarius is 4mg/kg (4 ppm).

Based on the regulatory limits there is the need to establish baseline information on the incidence of these toxins in some Ghanaian diets. This will help develop interventions to mitigate the toxins if present. This will help improve food security and food safety.

1.4 Objective

To assess the Fumonisin B_1 (FB_1) level in dried-maize, maize-dough and maize-flour from three major market centers in Kumasi; namely Asafo, Bantama and Kumasi Central market.

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize Production

It is globally known and determined that maize (zea mays) is an exceptional crop that is grown across a comprehensive range of agro ecological zones and is among the several forest crops planted from 7000 to 10,000 years which originated from the Central and South America (Ranum *et al.,* 2014). During the 16th century era, Portuguese traders introduced maize and it was comparatively the small crop in 1900 in Africa (Byerlee and Heisey, 1997). Maize together with rice and wheat are the most essential mankind food sources. According to the FAOSTAT (2012), consumption of all cereals is about 94% and also used for animal feed and biofuels. As an important crop, maize is a poor source of some nutrients such as iron, calcium and folate. It also lacks vitamin C and vitamins B12 but it provides vitamins B, fiber and some vital minerals (Ranum *et al*., 2014).

Protection of maize flour and maize dough(maize meal) with iron and other mineral and vitamins in countries and public health problems of severe and moderate anemia has helped curb iron deficiency and improve the intake of micronutrients (WHO, 2009). Although, maize is grown everywhere in Ghana, the forest-savannah zone is mostly suitable for its cultivation. This crop's cultivation is done by majority of smallholder-farmers in the rural areas in the country. Maize in Ghana today, is the most important cereal crop, which serves as commercial food for both local and foreign markets. Consumption of maize in Ghana is a wide variety such as porridges, grits and beers. The fresh maize is mostly eaten roasted or boiled as it plays a vital function in satisfying the humans after the dry seasons (ARC-Grain Crops Institute, 2003). However, in Ghana pre and post-harvest losses has resulted in the low yield of maize production by diseases, weeds and pests (Ofori and Kyei - Baffour, 2006). In 2005, maize under cultivation was 740,000ha and production was 1,171,000 metric tons by MOFA, 2006.

2.2 Mycotoxin

Fungi are microorganisms that produce Mycotoxins which are natural secondary metabolites. They are found in the soil, food, feeds as well as the environments (D'Mello and Macdonald, 1997). Many types of food harvests and food products are being infected by mycotoxins in the food chain (Turner *et al*., 2009; Reddy *et al*., 2010). Its infestation in crops mostly begin in the field, however once optimum temperature and moistures are maintained, there is continuation of the growth of these microorganisms through harvest, storage and food processing (Bhat and Vasanthi, 2003; Krska *et al*., 2008;Reddy et al., 2010). Insect invasion with humid conditions such as high temperatures, moisture, drought stress, during harvest lead to fungal increase and production of mycotoxin (Bhat and Vasanthi, 2003; D'Mello and Macdonald, 1997; IARC, 2002). The occurrence of both mycotoxins and fungal growth are as a results of these factors such as poor harvest practices, inadequate storage conditions and poor ways of handling food during transport to the market (Shephard, 2008).

Mycotoxins are among the most effective mutagenic and also cancer-causing substances known to man (Bhat and Vasanthi, 2003; Krska et al., 2008). Mycotoxins pose major health and economic concerns in many countries. Mycotoxins are largely classed into aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Aflatoxins are the most widely studied mycotoxins. They are carcinogenic byproducts of *Aspergillus flavus* and *Aspergillus parasiticus*. It comprises of four major groups which are aflatoxin B_1, B_2, G_1 and G_2. Its M_1 and M_2 are thermo – resistant hydroxylated metabolite produced by lactacting animals consuming feed contaminated by aflatoxin (Herzallah, 2009; Richard, 2000).

Afla B_1, B_2, G_1 and G_2 are known to be carcinogenic in humans and classified Group 1 whereas M_1 is probable carcinogenic in humans and classified as Group 2B (IoannouKakouri *et*

al.,1999). Fumonisins (FBs) are produced mainly in both maize and maize products mainly by *Fusarium verticillioides, Fusarium proliferatum* with fumonisins B1

(FB1) and fumonisins B2 (FB2) been the most abundant in naturally contaminated products.

FB1 and FB2 are classified as Group 2B potential carcinogen for humans (Wang *et al*., 2006). Reports by IARC, (2002) and Hussien *et al*., (2001) have associated FB_1 and FB_2 to the development of oesophageal cancer in humans in some areas in South Africa and parts of Asia.The effects of these toxins on both human health and animals tend to be very severe which could be carcinogenic, neurotoxic, mutagenic, immunosuppressive and also estrogenic (Krska *et al*., 2008; Bennett and Klich, 2003). Aflatoxins also tend to have adverse economic effects such as lesser yields for food (Sétamou *et al.,* 1997). Early childhood growth faltering could also be contributed by mycotoxins according to Turner *et al.*,(2003) with an apparent effect of initial exposure to aflatoxins. In West Africa, there has been studies showing epidemiological evidence of dose–response relation between aflatoxin exposure, the degree of stunting and underweight in young children under 5 years (Gong *et al*., 2002, 2003). Recently, hydrolyzed and bound forms have been identified in food products further complicating the problem of fumonisin contamination (Kim *et al*., 2003 and Park *et al.,* 2004). The TCA side chain on the fumonisin may be removed by treatment using alkaline. These toxins are quite heat stable and are mostly persistent through the conditions used in food processing yields hydrolysed analogues (hydrolysed fumonisin Bs - HFBs) (Murphy *et al.,*

1996).

2.2.1 Fumonisins in Maize

Fumonisins are produced by *fusarium* being one of the three genera of Mycotoxins: *Aspergillus, Penicillium.* Mycotoxins according to Patricia *et al* (2006) in their work refers to fungal metabolites which causes lowered performance, sicknesses and deaths in humans and animals when inhaled, ingested or being absorbed by means of the skin. Mycotoxins have been noted

to have had an adverse effect and greatly plagued human right from the inception of the production of crops. Matumba *et al* (2009) states that fumonisins are specifically a collection or class of metabolic toxic products by mold *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium nygamai* with *Fusarium verticillioides*; these are predominantly contaminant in maize. In 1998, Fumonisins had a premier quarantine, comprising of a long hydroxylated chain of hydrocarbons with additional tricarboxylic acid, methyl and amino categories or groups. These are long chain polyols esterified in C14 and C15 with tricarboxylic acids which are in two groups and are usually 20 carbons. Certainly, the main Fumonisins which are FB_1, FB_2, FB_3 happen to have natural occurrence as well as FA1 and

FA2 (Segvic and Pepeljnjaks, 2001). The 1993 classification of Fumonisins by International Agency for Research in Carcinogenesis (IARC) categorizes Fumonisins as 2B compounds which have a higher probability of being hazardous for humans (IARC, 1993). It was also revealed in the research that human diets contain much FB_1. Figures 2.1, 2.2, 2.3 and 2.4 indicate the various forms of fumonisin structures including structures for FB_1, FB_2 and FB_3.

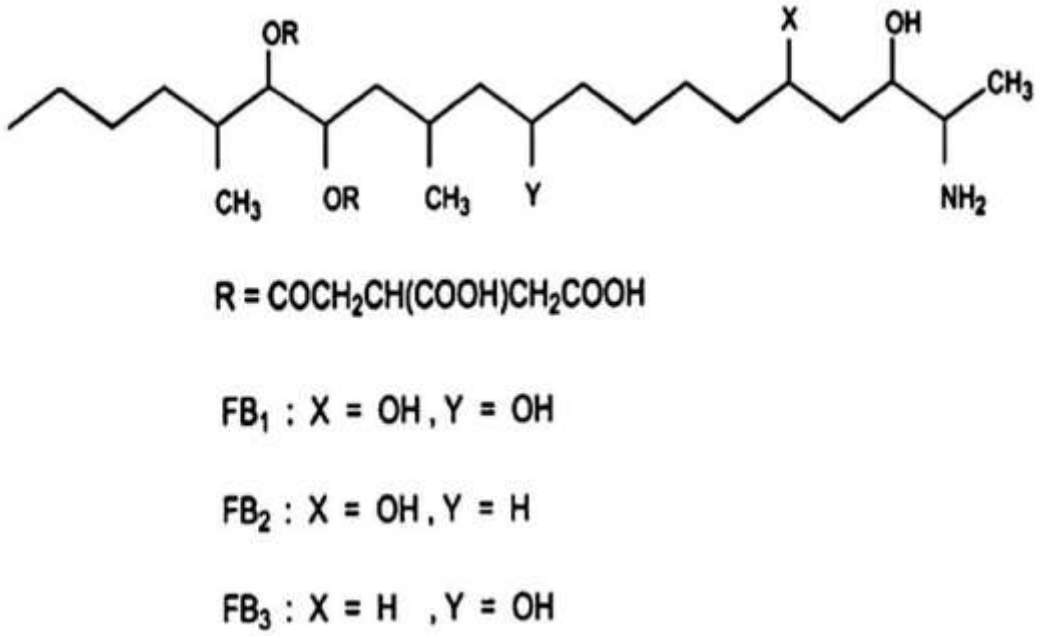

Figure 2.1: The Structure of Fuminosin

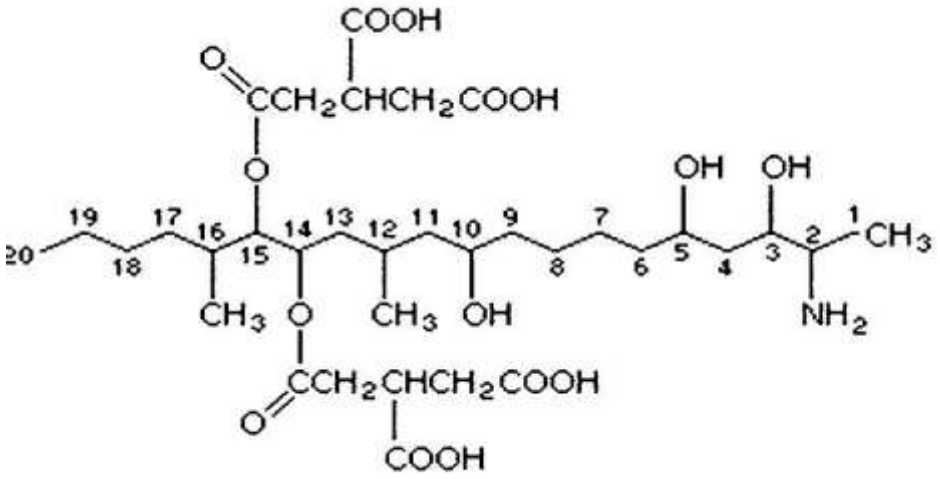

Figure 2.2: Structure of Fumonisin B1

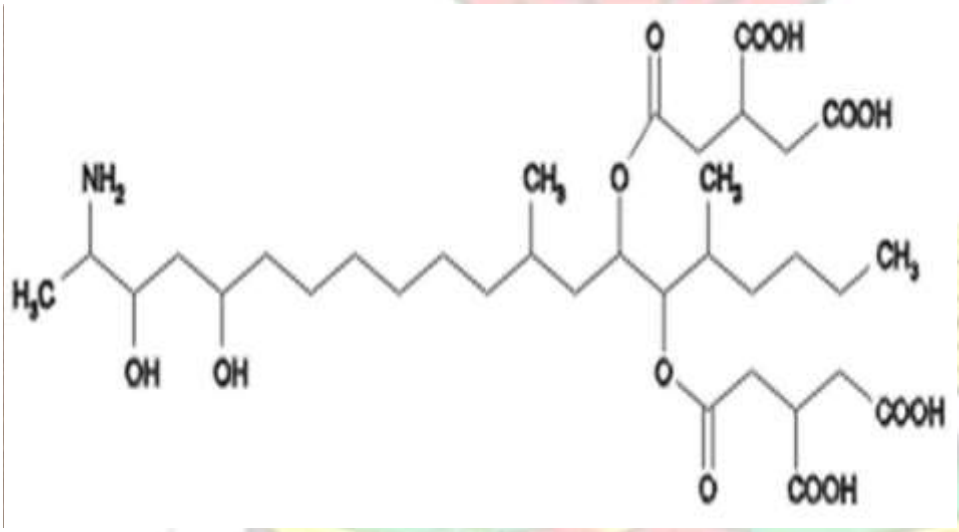

Figure 2.3: Structure of Fumonisin B2

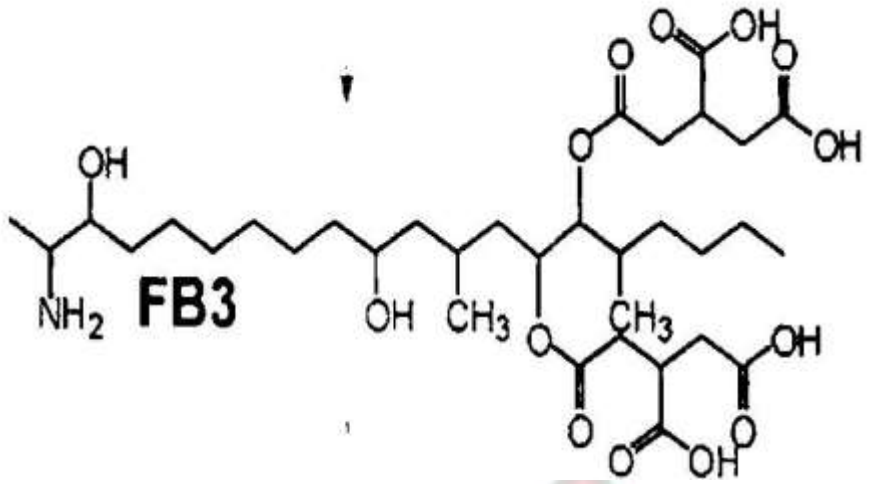

Figure 2.4: Structure of Fumonisin B3

Maize contamination of fumonisin is prevalent in numerous countries on the globe apparently 100,000µg/kg (100ppm) with respect to certain areas. In accordance with their research, the geographical location or area of a maize farm has a significant influence on the fumonisin contamination due to seasonal disparities and circumstances which characterizes the planting of a crop or grain and the growth thereof; the harvesting and storing of the crop is also affected by same conditions.

Comparatively, grain which is said to be grown in tropical and subtropical regions is mostly endangered by the contamination of fumonisins as a result of the relatively prolonged and warm season within which it was grown. In certain countries such as South Africa, the United States as well as China and Tanzania are said to have incremental hierarchies in relation to the fumonisin contamination of maize with rippling adverse effect on the country's economy and hazardous fauna (Matumba *et al*., 2009; Wild and Gong, 2010).

In terms of the toxicity of Fuminosins, Bhat *et al* (1997) FB1 is seen to possess the most toxins and prevalence in the family of Fuminosins. The common contaminant of maize which is fungus Fusarium *moniliforme* adversely affects the health of humans. Abdominal pains are

mostly as a result of the intake of moldy maize with the presence of FB_1 in certain parts of the world. The human esophageal cancer is a result of the consumption of maize that is contaminated. It is therefore revealed in accordance with Merril *et al* (2001) and Riley *et al* (1994), there is a close relationship between the births and thrive of diseases with associations with FB_1 and sphingolipid metabolism leading to impaired cycle of regulation of cell as well as cellular diversity.

In accordance with FAO (2014) the maximal allowed everyday consumption being standardized by FAO/WHO is said to be 2µg/kg body wt. per day FB_1 and FB_2 as well as FB_3. Voss *et al* (2007) and present statistical correlation showing that FB_1 and FB_2 greatly put the lives or health conditions of humans at risk and prevalent in the form of human esophageal cancer as well as cancer of the liver in many parts of the African Continent and Europe as well. It is also revealed by Choudhary & Kumari (2010), with regards to the scarcity of food or insufficiency, the populace in affected areas actually does not pay more careful attention to the choice of food quality or conditions under which crops were grown but rather go ahead into consumption to quench hunger or famine. It is approximately purged at 25% that the global food crops are then affected annually by mycotoxins and of most relevance is fumonisins.

2.3 The Possible Factors that Influences the Accumulation of Fumonisins

In accordance with Fotso *et al* (2002), fusarium species and fumonisins contamination affect cereals or grains and which are facilitated by various factors which encapsulate conditions of the environment within which the crop is grown as well as the infestation of insect either before or after harvesting. Various factors such as Environmental, Agricultural Practices, Cereal Characteristics, Post-Harvest Operations, Storage Insects and Fungi Interactions have various effects on maize and crop production in general.

2.3.1 The Environmental Factors

Globally, it is known in accordance with surveys conducted by various researchers and supported by Shepherd *et al* (1996) research, there are very high rises of fumonisins due to very warm as well as very dry climatic conditions. In this regard, fumonisins contaminate faster at the same locations but differ yearly in levels. A significant disparity in with reverence to contamination of fumonisins for particular maize differs within a dual season in a row; this is as a result of a discrepancy in environmental conditions. Working pressures within the time frame which leads harvesting of grain with respect to the weather in light of rainfall fluctuations as well as humidity relativeness; these premises further presents the best atmosphere for fumonisins to contaminate maize (Visconti, 1996). A very significant factor for the contamination of fumonisins has to do with the pre or post dryness of the weather of cereal pollination (Fotso *et al*., 2002).

2.3.2 Agricultural Practices

Within the wet or rainy conditions or seasons, cereal or grain cropping facilitates the growth of fumonisins and then is further, is predominant in the form of fungus which apparently is further increased in later seasons. It is known that mixed sowing and mixed cropping the identical crops presents the best podium for the haste infection of fungi and insect spasm on cereals. In the attempt to fighting the epidemic, the attempt to properly check weeds with which fumonisins possibly can be located further have an optimistic effect on the Fungal Infection; this then deals with the weeds which are non-host (Bilgrami and Choudhary, 1998; Al-Heeti, 1987).

2.3.3 Cereal Characteristics

Features of grain and cultivar of grains in terms of color, the composition of chemicals as well as developmental stages have adverse influence on the fungal infection and the making of

fumonisins. Cultivars that develop latter with declining moisture in grain eventually declines gradually less than 30%; due to this decline, they are virtually prone to the production of fumonisins (Marin *et al*., 1998; Fotso *et al*., 2002)

2.3.4 Post-Harvest Operations

Sorting and milling of harvested grains, fermentation and degree of favorability of forms of cooking, affects the production of fumonisins as well as infection of fungi in grains and cereals. Pre and Post-Harvest mechanical damage finds entrance into fungal spores in the cereals. It is well noted that, irrespective of the size of grains during processing, significant levels of increase in toxin are assured, most especially, small grains (Chandrashekar and Satyanayarana, 2006; Fotso *et al*., 2002). Figure 5 depicts some post-harvest practices of maize in Ghana.

2.3.5 Storage Insects

Insects are agents who wound and also act as vectors that spread fungi form the source of inoculum to the cereal plants. Insect wounds offer chances for fungal evasion of the natural cover of integument; in the end, they establish infectious sites on the inside which eventually becomes prone. The family Nitidulidae is borers and insects that greatly affect grains and cereals negatively by *fusarium* Species and fumonisins contamination. It is known that a significant increase of fumonisin pollution or contamination as a result of the infesting of *Busseola fusca.* These infections are then in maize cobs not taking in consideration if cobs are inoculated falsely with fungi (Velluti *et al*., 2000). Figure 5 depicts how s producers store the products.

2.3.6. Fungi Interactions

Maize that are harvested in tropical zones have the higher tendency of possessing mycelium as well as fungal species' spores, some of which are *penicillium* and *aspergillus* which usually

comes into agreement, grow and then are able to compete for food within favourable conditions within the environment which the crops or cereals are grown. It is known that *Fusarium verticillioides* and *Fusarium proliferatum* are the relevant fumonisin producers and are significantly reduced if *Fusarium graminearum* is present; in this case, production of fumonisin B_1 by the aforementioned would largely be repressed (Turjevic *et al*., 2005).

2.3.7 Incidence of Fumonisins B_1

Fumonisin B1 (FB1) is the most domimant (generally ~70% of the total fumonisin contamination), and it normally co-occurs with little traces of fumonisin B2 (FB2) and B3 (FB3). Occurrence in sorghum has also been reported (Bulder *et al.,* 2012). According to literature, maize-based foods and feeds are commonly contaminated with fumonisins in countries such as United States of America, China, Europe, South America and Africa (Shephard *et al.*, 1996; Visconti and Doko, 1994). Chilaka *et al*., (2016) analysed maize, sorghum, and millet selected from markets in four agro-ecological zones in Nigeria. Samples were assessed for fusarium mycotoxins. They reported that, 64% of the samples were contaminated with at least one toxin, at the rate of 77%, 44%, 59%, and 97% for maize, sorghum, millet, and *ogi*, respectively. Fumonisins were the most prevalent in maize and *ogi*, at the percentages of 65% and93% and their mean values as 935 and 1128 µg/kg, respectively. In Ghana, Kpodo *et al*., (2000) have isolated and determined the *fusarium* in some stored grains and cereals. Works by Kumi *et al*., (2014) showed that the levels of aflatoxin and fumonisins in foods made home reported the co-occurance of aflatoxin and fumonisin all 36 analyzed. Aflatoxin ranged from 7.9 to 500 µg/kg while fumonisin levels range from 0.74-11.0 µg/kg). 83.3% of the thirty-six samples exceeded the recommended limit of 20ppb for aflatoxin with a

mean of 145.2 ppb whilst 58.3% of the samples contaminated with fumonisins also exceeded the recommended limit of 4 ppm with a mean of 4.7 ppm.

2.4 Strategies of Controlling Fumonisins

There might be several strategies of controlling the contamination of fumonisins on grains or cereals with maize of no exception. The study would like to limit its control factors to Proper agricultural and manufacturing practices; Hazard Analysis and Critical Control Points (HACCP) and Biological control measures, transgenic approaches; Risk assessment and Food strategy regulations (Voss et al., 2001).

2.4.1 Proper Agricultural and Manufacturing Practices

The prior control of fumonisins is considered to be on the farm level which begins with the implementing and establishing of proper agricultural and manufacturing practices in other to preclude infections. Proper conditions under which maize is grown when planted such as testing of soil, conditioning of the field, rotation of crops and consistent irrigation; others of which are chemical treatments being antifungal, some of which are acetic acids and propionic. Other controls are largely preventing weed and insects. Some strategies and techniques for harvesting are said to comprise neat and very dry assortment and transporting of equipment, properly allocated conditions of harvesting; all the aforementioned strategies are relative to pre harvesting. In this regard, the post harvesting techniques or strategies may include the introduction of drying due to content of moisture of the maize already harvested, conditions under which maize is stored as well as the employment of carting vans of which must be free from fungal infection or growth (Quillien, 2002; Patricia *et al*., 2006). Both Figure 2.5 and 2.6 depicts some post-harvest processings.

Figure 2.5: Post-harvest Processing of Maize (Drying of maize)

Figure 2.6: Post-harvest Processing of maize (storage of maize)

2.4.2 Hazard Analysis and Critical Control Points

Postharvest control measures in the food industry against fumonisins contamination in accordance with Hazard Analysis and Critical Control Points comprise properly asserted structures supplier. This is said to present a conspicuous first-hand cover which eventually protects maize from being contaminated by fumonisins and on the other hand, pre harvesting sequencers are virtually being recorded in maize in various parts of Africa (Particia *et al.*, 2006; FAO/IAEA, 2001).

2.4.3 Biological Control Measures

The introduction of microorganisms for the detoxification of fumonisins has really been potentially capable in accordance with various researchers. Fumonisins contamination have actually be said to be declining in the field as well as in storage uninterruptedly. If was further proposed that the tendency of fumonisins contamination might have been metabolized by the enzymes of maize (Karlovsky, 1999).

2.4.4 Transgenic Approaches

Literatures by various researchers such as Karlovsky (1999) and Duvick (2001) shows transformation relative to mold susceptible genes foots vast potential for safety control; this has to do with antifungal proteins and metabolites which becomes a process of increasing compound production. Phenolics, hydroxamic acids and stilbeans declines infections caused by microorganisms by further introducing novel genes, expressing the target compound. On the same hand, engineering procedures in relation to genetics have the capability to increase enzyme production that would eventually degrade mycotoxins. It is well noted that transgenic maize are untested for fumonisin polluting maize for consumption. Patricia *et al* (2006) reveals that the reduction of insect adversely affecting plant in the form of injuries is an alternative procedure to minimizing fumonisin infection or contamination of maize since insects are major players of spread of mold in storage ad in field. Results obtained in this study add to the pool of data, which recognizes transgenic insect resistance as a deterrent of fumonisin contamination when maize is grown under conditions of significant infestation by Lepidopteran insects.

Overall, the presence of Bt genes effectively secured grain with significantly less insect injury and Fusarium ear rot compared to non-Bt, near-isogenic hybrids. In 2007, WBC infested plots were the most severely injured and hybrids susceptible to this insect (Cry1Ab hybrids and non-

Bt hybrids) were the most contaminated with FB1. FB1contamination was significantly lower in Cry1F hybrids, which expressed insecticidal. Total fumonisin

(FB1+FB) contamination was lower in Bt hybrids than in non-Bt hybrids for both ELISA and HPLC determinations. Total fumonisin contamination (FB1+FB2) +FB3 in maize obtained from Bt-hybrids (measured in 2008-2010) was below FDA guidance levels for even the most sensitive species in each year. In 2008, the average fumonisin level in non-Bt grain would have suffered market limitations as a result of its contamination, which exceeded 6 mg kg-1 as determined by HPLC and 11 mg kg-1 by ELISA (Sydenham *et al.,* 1990).

Manual insect infestations were used to ensure maize pest-populations were present in the field and that their distribution among plants was relatively uniform. Their presence increases the likelihood of fungal infection in maize tissues affected by feeding injury and fungal spore deposition throughout maize kernel tissues by active larvae. The choice of insect infestations was based on their traditional (ECB) and recent (WBC) significance in the growing area as well as the spectra of control provided by the Bt hybrids used in the study. In 2007, 2008, and 2009, the difference in kernel injury between naturally and artificiallyinfested plots was significant, indicating survival of manual infestations which likely exacerbated feeding effects on susceptible hybrids. This made differences among hybrids' susceptibilities to insects more apparent. Cry1F maize hybrids (Bt event TC1507) possess transgenic insect resistance, which is effective against both ECB and WBC. Both Bt hybrids were sufficiently protected against ECB infestation, but the susceptibility of Cry1Ab hybrids to WBC was evident. Feeding patterns differ between these two insects because WBC feeding was typically isolated at the tip of the ear and resulted in complete kernel destruction. ECB-feeding is generally more widespread throughout the ear; the larvae leave incomplete kernels in their path as they migrate through the ear. "Railroading" is also common in ECBinfested maize because small larvae move along a silk channel causing small amounts of injury to many kernels. While a strong

correlation was evident between insect injury and maize Fusarium ear rot severity (R=0.74), this may have been dampened because completely destroyed kernels were considered in the insect injury scores, but these kernels were not physically present to contribute to Fusarium ear rot scores or fumonisin contamination (Gelderblom *et al.*, 1991).

2.4.5 Risk Assessment

Various researchers have conducted studies on the assessment of fumonisins on the premise of experience of humans. In accordance with Kuiper-Goodman *et al* (1996) fumonisins are completely originated in maize as compared to other mycotoxins. It is well known that on the basis of almost 370 samples of food analysed over a cross section four years that, the fumonisin consumption by humans is as far as <0.089 µg/kg bw/d. It was then observed by the researchers that the consumption of fumonisins by humans were seemingly improbable to posing health dangers.

2.4.6 Food Safety Regulations

Karlovsky (1999) in his research asserts that, it is well noted that the knowledge of the public concerning the dangers surrounding mycotoxins is on the ascendency which is well to state that, quantification of toxin levels in advancing number quantum of food commodities by analytical chemistry is on the rise. It is also noted that transformed bioassays needed for the study of toxicology on certain defined areas or prone areas have seemingly exceed the capabilities of under classy methods which have eventually not allowed adverse dangers. On the same hand, the accessibility of monotonous procedures for testing efficiency and effectiveness as well as affordability has greatly provided for monitoring internal which has resulted in large sizes of contaminations and pollutions being well acknowledged.

2.5 Methods for Analysis of Fumonisins

It has been noted that the methods of analyses of fumonisins can include sampling as well as sub-sampling, suitable extraction as well as cleanup and concentration and even the derivatization prior to contributory analysis (Shephard *et al*., 2011). Cleanup is mainly needed and lays an integral part in the analysis of fumonisins as it removes the medium filths. Some cleanup methods include Immunoaffinity columns, Quick Easy Cheap Rugged and Safe, C18 columns which has its own merits.

2.5.1 Extracting Samples

The four carboxylic acid groups as well as the amine group make fumonisins very polar and then readily soluble in Polar Solvents. Which is further agreeable to the extraction in the process of the usage of methanol, acetonitrile, sodium dihydrogen phosphate which are all polar solvents and also applying diverse combinations and proportions prior to cleanup (Wilkes and Sutherland, 1998). It is then possible to predict an optimistic cleanup. It is well to not that food matrixes of food are properly extracted by acetonitrile or methanol with best outcome being anticipated when using methanol, water (3:1). On the other hand, efficiencies in extraction are being recorded due to the employment of blending or homogenization (Bennett and Richards, 1994). It is well noted that the introduction of very high solvents and sample ratios are said to create improvement in the recoveries of fumonisins and the extraction of highly polluted samples is seemingly uneasy as juxtaposed with the extracting spiked maize being attributable to the matrix composition or components within the sample under consideration.

2.5.2 Derivatisation

Researchers reveal that derivatisation enables for the very sensitive detection of fumonisins by the employment of spectrometric methods. It is then employed to form proper derivatives that would enable easy isolation and separation and then being detected. It is noted that the end result of the fumonisin analysis is being largely affected by the reagent used for the derivatisation (Sydenham *et al*., 1990).

2.6 Developments in Detection of Fumonisins

The employment of multi-mycotoxin analysis or assessment has provided for proper methods for fumonisins decontamination due to detection. Attention has been drawn within the toxicology industry with the employment of more sophisticated instruments such as UPHLC MS/MS and LC-MS/MS-ESI which have lately invited multifaceted procedures for simultaneous and concurrent detection of fumonisins and pesticides in maize (Gelderblom *et al*., 1991). A screening method proper for beverages, grains and feeds was introduces which contains chromatographic separation with MYCOTOXTM reversed phase C18 column and post-column separation instrument, Pinnacle PCX-Pickering Laboratories and an LC-MS/MS procedure multi-mycotoxin. Other increasingly screening procedures for fumonisins analysis has to do with fluorescence plarisation immune assay, lateral flow devices as well as biosensors (Bird *et al.*, 2002; Sulyok *et al*., 2010).

2.7 The Enzyme Linked Immunosorbent Assay (ELISA) Method

Fumonisin was analyzed in In 2007, there was fumonisin analysis with the use of quantitative ELISA method for fumonisin B_1 detection only. The Pioneer Hi-Bred Grain Analysis Laboratory (DuPont Pioneer, Johnston, IA), conducted this ELISA method and this was specifically for fumonisin $B_{1.}$ With this analysis, finely ground samples were used and

subsample of 3-g was extracted. ELISA is a constructed competition format with proprietary antibodies to fumonisin B1stabilized plates and were manufactured by Beacon Analytical Systems, Incorporated (Saco, ME, USA). Sample of maize extracts and horseradish peroxidase conjugated fumonisin B_1 and pre-coated as well as co-incubated of temperature of about 20-25°C and then shaken in the dark for 60-62 minutes. There was addition of substrates followed by washing of plates. Reaction of the substrates was then allowed to proceed and shaken for 30-32 minutes in the dark at 20-25°C. At 450 nm, resulting color intensities of the wells were read as the reaction was seized. Fumonisin B_1 amount was inversely proportional to the colour of the intensity in the assay well. 0.8 mg/kg to 2000 mg/kg was the measuring range for this ELISA method. This method has also been approved in comparison to HPLC by using AOAC-approved methods. There were determination of ground maize subsamples total fumonisin concentrations ($FB_1 + FB_2$) for each plot using AgraQuant Total +FB Fumonisin Assay 0.25/5.0 (Romer Laboratories Inc., Union, MO, USA) in 2008 and 2009. This is a direct-competitive ELISA efficient for quantifying fumonisins in solution at concentrations between 0.25 and 5 mg/kg. Appropriately, sample with fumonisin levels >5 mg/kg were diluted to be within the range of 10-g for the test. For samples containing fumonisin levels, the sample extract was diluted to bring it within range of the in10-g ELISA test. ELISA method is acceptable by United States Department of Agriculture - Grain Inspection, Packers & Stockyards Administration (USDA-GIPSA) for total fumonisins determination in ground maize.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Sampling of Maize, Maize-dough and Maize-flour

In Kumasi, maize, maize-dough and maize-flour is mostly consumed aside cassava and plantain. The aim of this survey was to sample maize and maize products such as maizedough and maize-flour for the determination of fumonisin B_1 level from three major market centers in Kumasi. A total of 63 samples of dried maize (n=22), maize-dough (n=21) and maize-flour (n=20) were collected from Kumasi Central Market, Asafo Market and Bantama Market in the month of June. A kilogram of maize, maize-dough and maize-flour were purchased from market sellers selected randomly from the three (3) markets. These markets were chosen because they are the major supplying centres of these commodities to nearby markets in the metropolis.

3.1.2 Chemicals used for extraction

All chemicals were purchased from Sigma-Aldrich, Inc., (Germany). Acetonitrile, Glacial acetic acid, Anhydrous Sodium chloride, Magnesium sulfate, Diatomaceous earth.

3.1.3 Derivatisation reagent chemicals and Preparation

Ortho-phthaldiaoldehyde (OPA), Sodium tetraborate and 2-Mercaptoethanol, Methanol. 40mg of the OPA was weighed and 1ml of methanol was used in dissolving the OPA . 5ml of 0.1M sodium tetraborate solution and 50µl of 2-mercaptoethanol were also added (Truckess, 2005).

3.2 Sample Preparation and determination of Fumonisin B_1

The sample preparation method used in determination of fumonisin B_1 and B_2 is according to (Petrarca *et al*., 2014). Samples purchased from the three markets were thoroughly mixed using

the Preethi Blender Mixer and 10g of each sample was weighed into 50 ml falcon polypropylene centrifuge tubes (Nalgene, Thermo Scientific, Rochester, NY, USA). In this method, 20 ml of 50% aqueous acetonitrile solution was added to 10g of each sample weighed into the tubes followed by the addition of 0.2 ml of glacial acetic acid and then vortexed for a minute. After vortexing, 2.5g of magnesium sulphate and then addition of 0.5g of sodium chloride to the mixtures and vortexed for a minute. The mixtures were then centrifuge at 7500rpm for 2 minutes. After this step, 5ml of the supernatant from each mixture measured into each 50ml polypropylene centrifuge tubes. A weight of 0.3g of magnesium sulphate and 0.1g diatomaceous earth were added and vortexed for 30 seconds and then centrifuged at 7500rpm for 2 minutes. Extracts from the mixtures were filtered through 0.22µm syringe filter and a precolumn-derivatisation reaction was done. The derivatisation reagent aliquot of 100µl was mixed with 100µl of the filtered extracts at room temperature, protected from light and within 3 minutes of adding the derivatisation reagent to the extracts, 50µl was injected into the HPLC (Truckess, 2005).

3.3 Conditions of Chromatography

The HPLC Cecil Adept Binary Pump with Shimadzu RF-10XL fluorescence detector and this was set at a excitation and emission wavelengths of 335 and 440nm, respectively. The column used was Supelco Discovery 150mm x 4.5mm, 5um and maintained at 40

Acetonitrile was used as the mobile phase with 0.1M sodium phosphate buffer solution (44:56) and then phosphoric acid used for adjusting the pH at 3.3. The run time was 20 minutes total run time and identification of fumonisin B_1 and B_2 according to Truckess, (2005) with comparison of both the retention time of the fumonisin B_1-OPA and fumonisin B_2-OPA derivative extracts and retention time of fumonisin B_1 and fumonisin B_2 standard.

3.4 Data Analysis

All the three commodities were performed in duplicate FB_1 analyses. Means were calculated with Minitab (State College, PA) statistical software. For the determination of the variations in the incidences of FB_1 levels in the maize products One-way analysis of variance (ANOVA) was used and then followed by Turkey test at 95% confidence. Graphs, tables and percentages were used to present the findings.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Incidence of Fumonisin levels in the three commodities

From the results of this work, fumonisin B_1 was found to be present in all food samples from the 3 markets. Maize-dough and maize-flour recorded 100% prevalence of fumonism B_1 while dried maize recorded 81.82% (Table 4.1). It can therefore be said that maize and maize products have high occurrence of fumonisin levels which have also been reported in studies by Kuiper-Goodman *et al*. (1996) and Park *et al*. (2002), Shephard *et al*. (1996), and Pohland, (1996),

Table 4.1: Incidence (%) of Fumonisin B_1 Levels

	Dried Maize	Maize-dough	Maize-flour
Number of Samples	22	21	20
FB_1 (% incidence)	81.82	100.00	100.00

Improper sorting before milling could be responsible for the higher incidence of fumonisin in the maize dough and flour as compared to the dried maize where grains can be handpicked to reduce contamination. Also, Bullerman *et al*. (2007) showed that when maize is not properly sorted out before they are processed into flour and dough there is a rise in concentration of mycotoxins.

4.2 Fumonisin levels in the three commodities at Asafo market

From the Asafo market, all maize products were contaminated with fumonisin, FB_1, at varying levels. Maize dough had the highest level of fumonisin, 24.08 mg/kg, which was followed by maize flour with 9.89 mg/kg and the least was found in dried maize, 0.55 mg/kg (Figure 4.1). This indicates that maize dough and flour from Asafo market are highly contaminated with FB_1 which exceed the European Union recommended limit of 4ppm by Codex Alimentarius (FAO, 2014). Only dried maize was found to be able to meet the standard. The high levels recorded

in the dough could be attributed to the wet milling process. Before wet milling, maize grains (both FB_1 contaminated and non-contaminated) are soaked overnight. After milling, fumonisins tend to be distributed in the fiber and germ (Bennet *et al*., 1996) hence the high levels of FB_1 in maize-dough.

In the production of maize flour, both contaminated and non-contaminated grains are milled together. There is no sorting of any kind in the traditional process of maize flour production either due to laziness or ignorance on the part of the producers. This will result in the high levels of fumonisin. For dried maize however, these are sorted before being sold on the market, since consumers will not buy contaminated or infected maize grains. This reduces the levels of fumonisins in dried maize as compared to maize dough and flour.

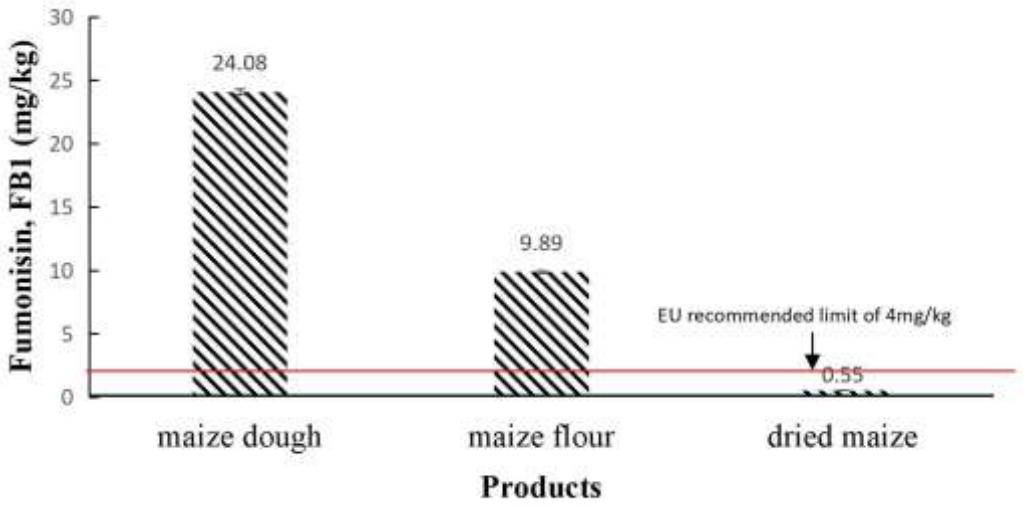

Figure 4.1: Fumonisin Level of Maize and Maize Products from Asafo Market

4.3 Fumonisin levels in the three commodities at Bantama market

Fumonisin levels in the maize products in Bantama market were also observed to be very high, except for dried maize. However unlike Asafo market, maize flour had the highest level of fumonisin, 12.07 mg/kg while maize dough had 1.99 mg/kg (Figure 4.2). This variation may be due to the source of maize for the maize flour and dough. The grains used for the maize flour may have been more contaminated than that of the dough. Maize processors often get their

maize grains from different sources but may end up mixing them before processing. Therefore, it is often difficult to trace the source of maize. Moldy grains due to bad postharvest handling methods such as improper drying and storage could result in high aflatoxin and fumonisin levels.

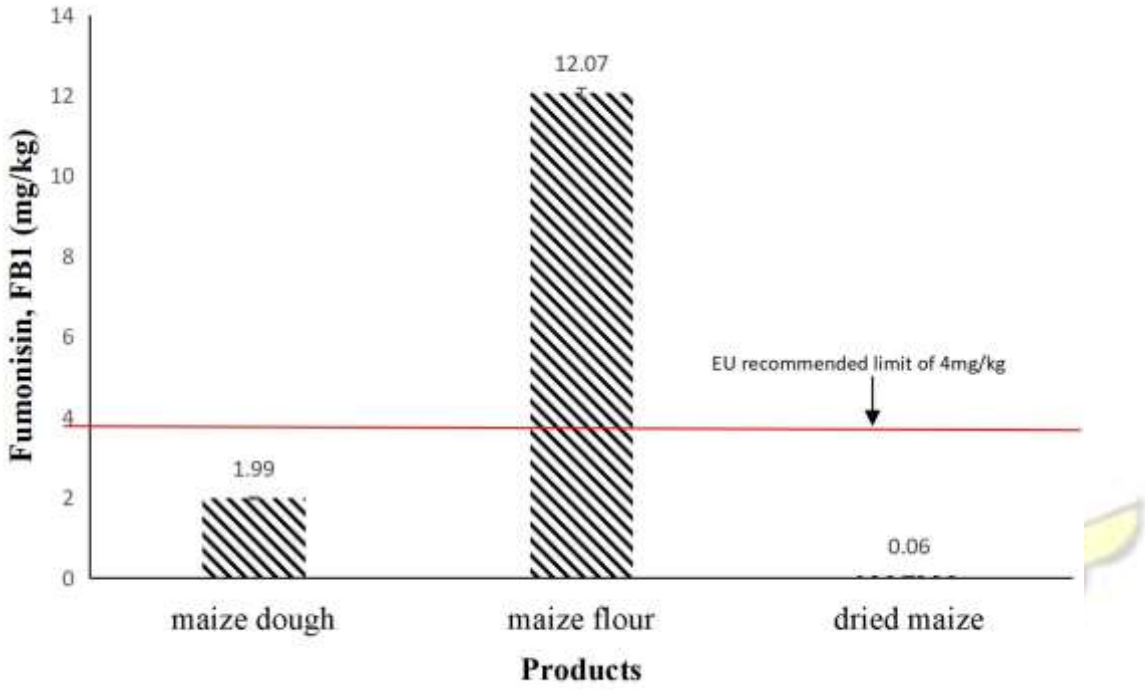

Figure 4.2: Fumonisins Level of Maize and Maize Products from Bantama Market

The levels of FB_1 in both maize dough and dried maize were below the EU recommended limit of 4 mg/kg. According to Brera *et al.*, (2004), there is a distribution of fumonisin B_1 in maize fractions during dry milling. Also, poor storage of maize flour causes mold infestation which is not usually visible hence contaminating the entire product when mixed by most sellers in the Bantama market. The flour usually is moldy due to ineffective drying.

4.4 Fumonisin levels in the three commodities at Kumasi Central Market

As was observed at the Bantama market, maize flour samples had the highest fumonisin level, 9.67mg/kg, which was higher than the EU recommended level, 4mg/kg. Maize dough and dried maize from this market had relatively lower levels, 2.58mg/kg and 0.84mg/kg, respectively (Figure 4.3). These variations could be attributed to the source of maize grains before being processed into dough and flour.

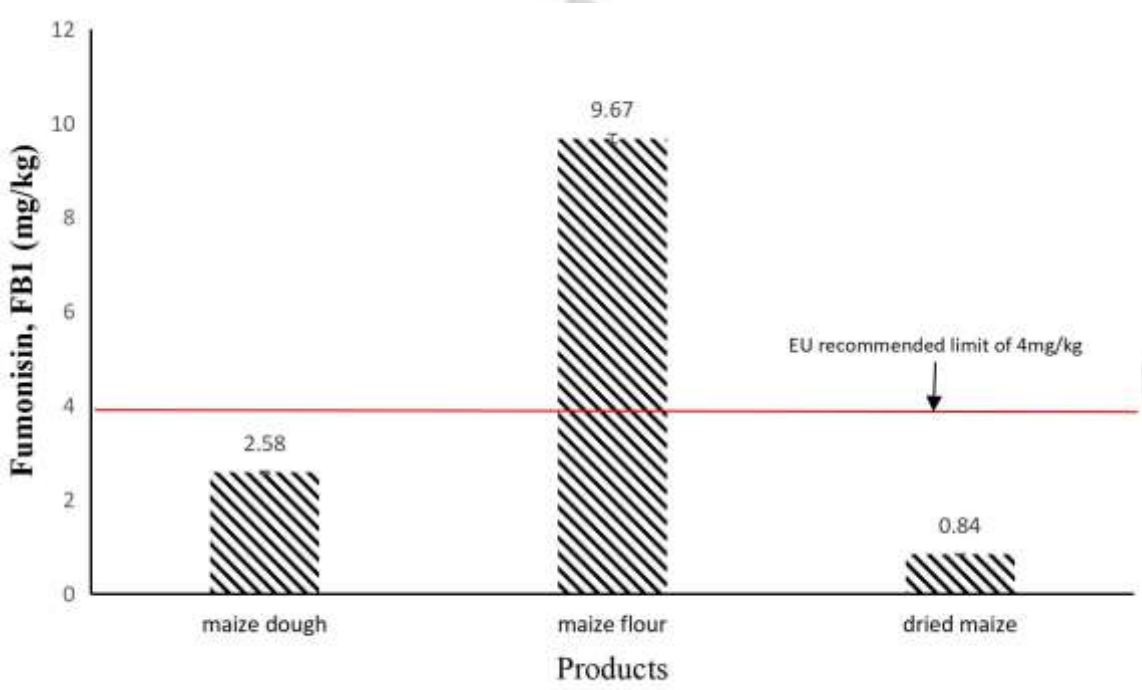

Figure 4.1: Fumonisin Level Maize and Maize of Products from Central market

Amongst the maize dough samples, it was observed that samples from the Asafo market had the highest fumonisin (FB_1) level, with that of Bantama market having the least. Maize flour from Bantama market had the highest level of fumonisin while that of Kumasi Central market had the least. Dried maize had the least of fumonisin levels amongst all maize products in all three markets. Their levels were far lower than the limit set by the EU. The low levels in dried maize from all three markets could be as a result of its storage in airtight jute bags and low

moisture content, which prevents invasion of insects, mold growth and also high temperatures. Occasionally, these markets sellers remove moldy grains, insect damaged and broken grains by hand which also accounts for the low FB_1 level in dried maize. Therefore this commodity could be encouraged for consumption by both humans and domesticated animals.

Consumption of the contaminated maize products is dangerous to domesticated animals and human as FB_1 has been proven as a cause of esophageal and liver cancer in humans (Sydenham *et al*., 1990; Yohizawa *et al*., 1994; Missmer *et al*, 2006 Shephard *et al* 1996; Pohland, 1996; Kuiper-Goodman *et al*, 1996 ;Park *et al*, 2002).

4.5 Average Fumonisin levels in the maize products from the three markets

When all markets were put together to ascertain which maize product or commodity had the highest fumonisin content and whether or not these were significantly different from the rest, it was observed that maize-dough had the highest fumonisin B_1 level, 12.19 mg/kg (Table 2). This value was found to be significantly different from dried maize which had the least of fumonisin B_1, 0.62 mg/kg (Table 4.2). The level for maize-dough however was not significantly different from maize flour, which had 10.83 mg/kg (p-value = 0.0387).

Table 4.1: Levels of fumonisins in the commodities from the three markets

Commodity	Fumonisin B_1 (mg/kg)
Dried maize	0.62^a
Maize-dough	12.19^b
Maize-flour	10.83^b
p-value	0.0387

The findings from the survey indicate the incidence of FB_1 contamination in maize and maize products sold on bigger markets in the Kumasi metropolis. It also shows that less is being done

in relation to the prevention or control of FB_1 in maize and maize products hence making its consumption a threat to the health of consumers.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Maize-dough and flour in the Asafo market were found to have the highest levels of FB_1 (24.08 mg/kg and 9.89 mg/kg, respectively) and dried maize had the least (0.55 mg/kg). At the Bantama market, maize-flour had the highest FB_1 level (12.07 mg/kg), which was much higher than that of maize-dough (1.99 mg/kg) and dried maize (0.06 mg/kg). Also, Kumasi Central market followed a similar trend as for Bantama with maize-flour having the highest level of FB_1 (9.67 mg/kg), maize-dough and dried maize had 2.58 mg/kg and 0.84 mg/kg, respectively. Levels of FB1 in maize-dough and maize-flour was 6x and 2.5x higher than the EU recommended limit of 4ppm in the Asafo market respectively. Also, levels of Fbi of maize-flour from Bantama and Kumasi Central Markets was 3x and 2.5x higher than the EU recommended limit of 4ppm respectively.

5.2 Recommendations

It is recommended that FB1 levels in maize products in other markets across the country should be assessed in addition to the risk assessment of fumonisins in Ghana.

The following points are also recommended for consideration in order to help curb the problem.

Research and Regulation

Policy makers which include FDA and GSA should give more attention to fumonisins B_1 just as is done to aflatoxins in the country. There should be extensive research on fumonisins B_1 to help in knowing the tolerable limit intake as it is the most prevalent one. There should also be

continued surveillance of fumonisin B_1 on maize and its products frequently as it may vary from market to market with time.

Education

Farmers should be educated on pre-harvest and post-harvest practices on maize to minimize the mycotoxin contamination. On the other hand, sellers of maize products should also be educated on how to store the products in order to minimize contamination which will affect the health of final consumers.

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