KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

DISC ATTRITION MILL (CORN MILL) AS A MEDIUM FOR AFLATOXIN CONTAMINATION IN MILLED MAIZE

A THESIS SUBMITED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF

MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

BY

AGBEVE NELSON KWASI

(BSc. Hons)

March, 2016

DECLARATION

I hereby declare that the submission of this manuscript is the true findings of my own research work for the award of an MSc. in Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor submitted to any other university or institution for the award of degree except where due acknowledgement has been made in text. However, references from the work of others have been clearly stated.

……. …….

Agbeve Nelson Kwasi Dr. Herman E. Lutterodt

(Student) (Supervisor)

Date:…… Date:……..

DEDICATION

This manuscript is dedicated to the Almighty God; my parents, Mr. and Mrs Agbeve; My supervisor, Dr. Herman E. Lutterodt; Mr. Kofi Essel, head of food Industrial Services Support Department of the Food and Drugs Authority and Mr Solomon Agampim, head of Import and Export Control Department of the food and Drugs Authority.

ACKNOWLEDGEMENT

I would like to use this opportunity to show my profound gratitude to the following personalities:

My deepest thank goes to my supervisor, Dr. Herman E. Lutterodt of the Department of Food Science and technology, Kwame Nkrumah University of Science and technology for having sacrificed his time to read through this manuscript several times to make the necessary corrections.

I equally thank Mr. William Appaw Offori, head of Mycotoxin Laboratory of Kwame Nkrumah University of Science and Technology for devoting his time to analyze the numerous samples that were gathered from the Kumasi metropolis and also helped me acquire practical knowledge in the realisation of this project work.

My appreciation also goes to Mr. Kofi Essel, Principal Regulatory Officer at the Food and Drugs Authority for having made a vital input in the choice of topic.

My final appreciation goes to the university authorities for having made available the laboratory for my analysis.

ABSTRACT

Two (2) disc attrition mill popularly known in Ghana as “nika nika” were selected from two sub metros of different socioeconomic status (Low income earners and High income earners) in Kumasi. They were audited against good manufacturing practices (GMP) checklist. Maize samples were purchased from the market at each sample point. They were filled into low density polyethylene (LDPE) zip lock bags and wrapped in aluminum foil. They were distributed to the four corn mill facilities in an ice chest filled with ice in the morning and peak period of the day of maximum and minimum sale at each sampling point. The samples and the controls were analyzed for aflatoxin. The four disc attrition mills scored below 25 % after the GMP audit. This was an indication of poor hygienic conditions that existed. The data obtained from the laboratory was analyzed. The aflatoxin levels of the maize sold in the market significantly exceeded the maximum acceptable level in Ghana with a $p < 0.05$. Samples milled in the morning and the peak of the day of maximum sale showed significant increase in the level of aflatoxin with $p < 0.05$. This increment may be attributed to the fact that, the test samples had contact with previously milled products that were contaminated with aflatoxin as well as remnants that got stuck in the grinding discs. A $p > 0.05$ showed that there was no significant increase in the level of aflatoxin in the test samples milled in the morning and peak period of the day of minimum sale. The test samples that were milled by disc attrition mills located in the low income earners communities were significantly higher than those in high income earners ($p < 0.05$). This implied that, clients who patronize disc attrition mills at the low income communities were at higher risk of aflatoxin contamination of their samples than those in the high income earners communities.

Key words: disc attrition mills, aflatoxin, good manufacturing practices

RESUMÉ

Deux (2) moulin à maïs à commerce populairement connu au Ghana comme " nika nika " ont été choisis dans deux sous métros de niveau socioéconomique différent (la classe inférieure et la classe supérieure) à Kumasi. Ils ont été vérifiés contre le principe de bonnes pratiques de fabrication (BPF). Des échantillons de maïs ont été achetés au marché à chaque point d'échantillonnage. Ils ont été introduits dans des sachet de verrouillage de polyéthylène basse densité (PEBD) et enveloppé dans une feuille d'aluminium. Ils ont été distribués aux quatre installations commercial de moulin à maïs dans un coffre glacé pendant le matin et l'heure de pointe de la journée de vente maximale et minimale avec un témoin stocké à chaque point d'échantillonnage. Les échantillons ont été analysés pour l'aflatoxine. Les quatre usines commerciales étaient en dessous de 25 % après la vérification. Cela a été une indication de mauvaises conditions d'hygiène qui existaient. Les données obtenues au niveau du laboratoire ont été analysées. Les niveaux de l'aflatoxine du maïs vendu au marché ont largement dépassé le niveau maximale acceptable au Ghana avec une valeur de $p < 0,05$. Les échantillons broyés pendant le matin et l'heure de pointe de la journée de vente maximale ont montrés une augmentation significative de la teneur en aflatoxine avec une valeaur de $p < 0,05$. Cette augmentation peut être expliqué que, les échantillons d'essai ont été en contact avec des produits préalablement broyées qui ont été contaminés par l'aflatoxine, ainsi que des restes qui est resté coincé dans la lame de broyage. Une valeur de $p > 0,05$ a montré qu'il n'y avait pas d'augmentation significative dans le niveau de l'aflatoxine dans les échantillons d'essai broyés pendant le matin et l'heure de pointe de la journée de vente minimale. Les échantillons d'essai qui ont été broyés par les moulins aux maïs commercials situées dans les communautés de la classe inférieure étaient significativement plus élevés que ceux dans les communautés de la classe supérieure ($p < 0,05$). Cela impliquait que, les clients qui fréquentent les moulins aux maïs

commercials aux communautés de la classe inférieure étaient plus à risque de contamination par les aflatoxines de leurs échantillons que ceux dans les communautés de la classe supérieure.

Mots clés: moulins commerciaux, l'aflatoxine, les bonnes pratiques de fabrication

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AFB_1	:	Aflatoxin B_1
AOAC	:	Association of Analytical Communities
A*w*	:	Water Activity
BPF	:	Bonnes Pratiques de Fabrication (BPF)
CTN	:	Citrinin
DNA	:	Deoxyribonucleic Acid
EFSA	:	European Food Safety Authority
FDA	:	Food and Drugs Authority
GMP	:	Good Manufacturing Practices
GNA	:	Ghana News Agency
GSA	:	Ghana Standard Authority
GSS	:	Ghana Statistical Service
HPLC	:	High Performance Liquid Chromatography
ISSD	:	Industrial Support Services Department
KMA	:	Kumasi Metropolitan Assembly
KNUST	:	Kwame Nkrumah University of Science and Technology
LD	:	Lethal Dose
LDPE	:	Low-density polyethylene
LLDPE	:	Linear Low-density polyethylene

OTA : Ochratoxin A

PEBD : Polyéthylène Basse Densité

TFA : Trifloroacetic acid

UV : Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Aflatoxins are mycotoxins that were discovered around the 1960's. This discovery was as a result of the knowledge that, the toxins of *Aspergillus flavus* was the cause of 'Turkey X disease' that caused numerous death in poultry in Britain (Wannop, 1961). Abbas *et al.* (2004) listed *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* as the fungi that produce mycotoxins as their metabolites. The maximum acceptable limits of mould and aflatoxins for degermed maize (corn) meal and maize (corn) grits in Ghana are 1 x 10^4 cfu/g and 20 ppb respectively (Ghana Standard, 2003). Chronic human exposure to aflatoxin in high quantity results in cirrhosis.

There exist about 14 different types of aflatoxins in nature (Boutrif, 1998). But aflatoxin B_1 is considered the most potent cancer causing mycotoxin among them (Martins *et al.*, 2001; Yin *et al.*, 2008). A study by Shivender and Anupreet (2012) on the Toxicity, characteristics and analysis of aflatoxin B_1 indicated oral $LD_{50\ (oral,\ duckling)}$ and $LD_{50\ (oral,\ mouse)}$ of Aflatoxin B_1 as 0.43 mg/kg per bodyweight and 9.0 mg/kg per bodyweight respectively. Others include aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin M_1 (AFM_1) and aflatoxin G_2 (AFG_2).

Mycotoxins are believed to be irrelevant for the growth of the mould and that the reason for their production is not really known (Fox and Howlett, 2008). In view of this they are termed secondary metabolite. About 4.5 billion persons living in developing countries are chronically exposed to largely uncontrolled amounts of the toxin (Williams *et al.*, 2004). In a research conducted by Kumi

et al. (2014), children fed on weaning food were being exposed to high levels of aflatoxin and fumonisin in the Ejura- Sekyedumase district in the Ashanti Region.

Aspergillus flavus colonizes maize, oil seeds, spices, groundnuts, tree nuts, milk and dried fruit (Strosnider *et al.,* 2006), however the synthesis of aflatoxin depends on relative humidity, suitability of crop genotype for its climate, insect damage, and agricultural practices (Wu and Khlangwiset, 2010) as well as "postharvest" conditions such as storage, transportation, and food processing. Maize and peanuts are highly consumed in the world and are also the main sources of human exposure to aflatoxin (Wu and Khlangwiset, 2010).

The aged, children and the immunosuppressed are more susceptible (Williams *et al.*, 2004). Children are more susceptible due to their physiology, lack of food diversity, and a higher consumption relative to their body.

Legumes, nuts, vegetables and pulses, and cereals such as rice, wheat, corn and barley are one of the most important sources of food in Ghana. Maize is Ghana's number one staple crop followed by rice, and domestic demand for both is high. The compound annual demand is pegged at the rate of 2.6 % resulting in a short fall of domestic supply of 12 % (Armah, 2010).

In Ghanaian communities, maize undergoes different forms of processing and one of the most common ways is to subject them to milling using disc attrition mills popularly known as "nika nika" or corn mill (Achiaa, 2015). They are milled into flour and made into dough by individual households and small scale manufacturers. However, improper storage or processing of the product may result in the growth of moulds and subsequently contaminated with aflatoxin and may then cause aflatoxicosis in consumers of these products.

The maize may not only get contaminated with aflatoxin, but also with iron filings during milling. During milling, iron particles from grinding discs contaminate the maize flour and according to Kwofie *et al*. (2011) the level of contamination increases with increasing quantity of maize milled. This may pose possible health threat to the consumers of foods prepared from such contaminated milled maize.

Chemical and physical hazardous substances such as paints, grease, wood, pieces of broom as well as pieces of hair may also be found in the food.

1.2 PROBLEM STATEMENT AND JUSTIFICATION

A pre-assessment of the corn mills in Accra metropolis reveals that 21 % failed good manufacturing practices (GMP), 77 % were operating under very poor hygiene conditions and 2 % were operating under appreciable level of hygienic conditions (Essel *et al*., 2015), and these conditions may pose a serious threat to food safety.

During the milling process, residues of the product get stuck on the grinding disc and when left in there for hours and days, it may result in the growth of moulds which may lead to the production of aflatoxins. In addition, products milled may be pre-contaminated with moulds and aflatoxins. The residue if not completely removed may contaminate the subsequent product to be milled from clients which may be aflatoxin free. The end product will eventually reach consumers which poses serious health threats because unlike microbial contamination, aflatoxin cannot be destroyed by cooking. The disc attrition mill may therefore be considered as a medium of microbial and aflatoxin contamination. Quality control measures of the raw material to be processed is either minimal or completely non-existent at the facilities and so affect the quality of the final product.

In as much as the above observation may be obvious, its effect cannot be concluded to be significant without passing through well laid down scientific procedure, documenting the significance of the milling process in contributing to the already high aflatoxin contamination problem.

This sector is under the supervision of the district and municipal assembly under the Ministry of Local Government and Rural Development, but inadequate human and other resources at the municipal assembly result in poor monitoring of hygiene and sanitation. And also, visit to the facilities by officers of the local government is more of revenue collection than hygiene audit.

There is high patronage of the commercial mill by the Ghanaian populace. About 70 % of small to - medium scale food manufacturers patronize the services of disc attrition mills (FDA, 2014), indicating that majority of the Ghanaians may be at risk of aflatoxicosis including infants, and other vulnerable people of the population if less attention is given to it.

The absence of empirical data on the contribution of the operations of disc attrition mills to public health as may be responsible for the less attention given to the industry in regulatory frameworks. This project will provide enough scientific documentary evidence to the municipal assembly under the Ministry of Local Government and

Rural Development and other regulatory agencies like the FDA to formulate regulatory policies to streamline and monitor the activities in this sector.

1.3 OBJECTIVES

The objectives of this study were:

1. To assess the hygiene situation in commercial milling facilities in the Kumasi Metropolis
2. To determine the extent of contribution of the commercial milling facilities to aflatoxin contamination of milled maize.

CHAPTER TWO

LITERATURE REVIEW

2.1 MYCOTOXINS

Mycotoxins are toxins produced by species of organisms classified under the kingdom fungi. They are by-products of fungi that have undergone metabolism and they are toxic. The efficacy and the potency of the toxins produced depends on the environment in which the fungi are found which can either be intrinsic or extrinsic because the susceptibility, rate of metabolism and defense mechanism differ from host to host (Hussein and Brasel, 2001). Exposure to mycotoxin poses serious health threat to humans including death, weakened immune systems without specificity to a toxin.

2.1.1 Aflatoxins

Aflatoxins are mycotoxins that were discovered around the 1960's. This discovery was as a result of the knowledge that, the toxins of *Aspergillus flavus* was the cause of 'Turkey X disease' that caused numerous death of poultry birds in Britain (Wannop, 1961). They are series of bis furan polycyclic compounds which are not only potent acute toxins in several species, but also known potent hepato carcinogens (Brase *et al*., 2013). They are not destroyed by cooking, however they are generally unstable when exposed to ultraviolet radiation and fluorescent lamps. Aflatoxins are one of the mycotoxins among many produced by certain species of moulds. These species include *Aspergillus flavus* and *Aspergillus parasiticus* (Martins *et al*., 2001). Moulds can grow in unfavourable conditions such as low water activity *(Aw)*, high osmotic pressure and low pH. It is therefore almost practically impossible to avoid the contamination of cereal products with aflatoxin. So regulators of food products have developed standards to establish a maximum acceptable limit. These limits may vary slightly from country to country. The maximum acceptable

limit of mould and aflatoxins in Ghana for degermed maize (corn) meal and maize (corn) grits are 1×10^4 cfu/g and 20 ppb respectively (Ghana Standard, 2003).

There exist about 14 different types of aflatoxins in nature, synthesized by mould species (Boutrif, 1998), but aflatoxin B_1 is considered the most potent cancer causing mycotoxin and among the diseases is cirrhosis (liver cancer) (Yin *et al.*, 2008; Martins *et al.*, 2001). A study by Shivender and Anupreet (2012) on the Toxicity, characteristics and analysis of aflatoxin B_1 indicated oral $LD_{50\ (oral,\ duckling)}$ and $LD_{50\ (oral,\ mouse)}$ of Aflatoxin B_1 as 0.43 mg/kg per bodyweight and 9.0 mg/kg per bodyweight respectively. Aflatoxin B_2 is a derivative of aflatoxin B_1 and has lower toxicity with $LD_{50\ (oral,\ duckling)}$ being 1-70 mg/kg (Purchase, 1967). Other forms of aflatoxins include aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin M_1 (AFM_1) and aflatoxin G_2 (AFG_2).

Mycotoxins may be necessary for the survival of the mould but irrelevant for their growth and the reason for their production is not really known (Fox and Howlett, 2008). In view of this they are termed secondary metabolites. It is also generally believed that aflatoxin B_1 is the precursor for the other aflatoxins (Thomas, 1965; Biollaz *et al.*, 1970).

2.1.1-1 Factors affecting aflatoxin production

The production of mycotoxins are dependent on the conditions of the medium in which the mould is located. *Aspergillus flavus* produce aflatoxin in maize when the grains grow under harsh and stressful conditions such as drought (Milani, 2013). The extent of growth of aflatoxigenic mould as well as the level of aflatoxin occur through an integration of environmental factors. Storing the grains for about two to five years contributes to mould growth as well as aflatoxin production under certain conditions (Obioha, 1979). These conditions include high moisture content of the cereal grain about 13 %, high temperature (75 - 95^o F), time (7 - 15 days), insect damage and physical

condition of the grain (weathering, mechanical handling) (Christensen and Kaufman, 1965; Anonymous, 1967). It was established by Ellis *et al.* (1993) that water activity (A_w), pH, storage temperature and initial concentration of headspace are all highly significant factors in controlling the growth of *A. flavus* and aflatoxin levels on synthetic media and thus aflatoxin production increases with increasing colony diameter.

2.1.1-2 Types of aflatoxin and their characteristics

Aflatoxin B_1

Among all the aflatoxins, aflatoxin B_1 is considered the most potent which is associated with liver cancer (Martins *et al.,* 2001; Yin *et al.,* 2008). It can penetrate through the skin, and dermal exposure to it in the environment can lead to major skin problems (Boonen *et al.,* 2012). It is hepatotoxic, mutagenic, carcinogenic and teratogenic. It is a colourless to pale yellow crystals or white powder that exhibits blue fluorescence. It is sensitive when exposed to air and light and also water insoluble. It is incompatible with strong oxidizing agents, strong acids and strong bases but probably combustible. *Aspergillus flavus* produces only Aflatoxin B_1 but *Aspergilus parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2 (Horn *et al*., 2009).

Aflatoxin B_2

It is a blue-fluorescent metabolite of *Aspergillus flavus*. It was isolated from cultures grown on crushed wheat. Chemical structure of the compound was explicated as dihydroaflatoxin B (1). When ducklings were exposed to it, they showed symptoms of reduction in growth and liver size and an extent of bile-duct hyperplasia (Chang *et al*., 1963). It has a chemical formula of $C_{17}H_{14}O_6$ and a molar mass of 314.29 $g \cdot mol^{-1}$.

Aflatoxins G_1 and G_2, M_1 and M_2

Aflatoxins G_1 and G_2 emit green florescence but aflatoxins M_1 and M_2 emit blue florescence when exposed to UV light. Aflatoxins M1 and M_2 were discovered in the milk of cows that fed on mouldy grains and are metabolites of Aflatoxins B_1 and B_2 respectively.

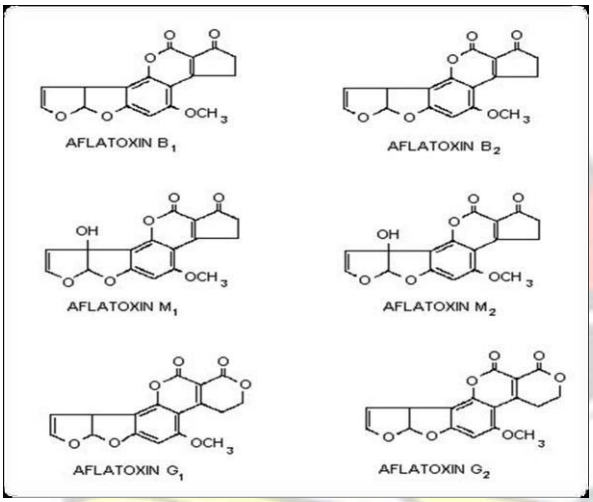

Figure 1: Structural representation of the six most occurring aflatoxins

The physical parameters of the different types of aflatoxin indicating their molecular formula, molar mass, melting points and their maximum ultraviolet absorption limits are shown in Table 1 below:

Table 1: Properties of common aflatoxin

Type of aflatoxin	Molecular formula	Molar mass	Melting point	UV absorption max (e), nm, methanol	
B_1	$C_{17}H_{12}O_6$	312 g·mol^{-1}	267(d)*	12,400	21,800
B_2	$C_{17}H_{14}O_6$	314 g·mol^{-1}	303-306(d)	12,100	24,000
G_1	$C_{17}H_{14}O_6$	328 g·mol^{-1}	257-259	9,600	17,700
G_2	$C_{17}H_{14}O_7$	330 g·mol^{-1}	237-240	8,200	17,100
M_1	$C_{17}H_{12}O_7$	328 g·mol^{-1}	299(d)	14,150	21,250
M_2	$C_{17}H_{14}O_7$	330 g·mol^{-1}	293(d)	12,100	22,900

Source (Fact sheet: www.micotoxinas.com.br)

2.1.1-3 Effect of some electromagnetic radiations on Aflatoxin levels

Ultra violet light and fluorescent light have the ability to reduce the toxicity of a more potent mycotoxin to a less potent one or inhibit the production of mycotoxins in wheat grains. This has been studied and documented by several researchers. When a fungal isolate known to produce mycotoxins were grown on wheat grains and briefly exposed to fluorescent light with exposure to ultra violet light for a long time, under relative humidity of 50 % to 80 % at room temperature, some mycotoxins were totally eliminated while others varied in their concentration depending on the relative humidity and period of illumination (Atalla *et al*., 2004). This confirmed that UV and fluorescent lights have substantial effect on the levels of aflatoxin in grains. The gas from the ozone layer and the radiation from the microwave also have appreciable effect on the levels of moulds and mycotoxins in grains. A work done by Halima *et al*., (2015) on the treatment of fungal infected grain and the reduction of mycotoxin in grains with exposure to ozone gas indicated that the optimum concentrations in achieving maximum reduction was 2 g/min for 2 min, the optimum time exposition to UV light were 140 min at 240 nm wavelengths and 60 min at 365 nm wavelength and finally the optimum period of exposition to microwave is 5min with 0.12 g and 0.21 g biomass.

The UV light method is one of the simple techniques used to screen and detect grains contaminated with aflatoxin. Under UV radiations, grains that may be contaminated with aflatoxin may emit blue or green fluorescence. If no fluorescence is observed, aflatoxin is probably not present in the grain in excessive quantities. When the simple UV light proves positive, one may further go on with confirmatory test using Column chromatography, Thin layer Chromatography or High Performance Liquid Chromatography.

2.1.1-4 Aflatoxicosis in humans

Aspergillus flavus and *A. parasiticus* colonize the following food commodities maize, oilseeds, spices, groundnuts, tree nuts, milk, and dried fruit (Strosnider *et al.*, 2006). But the ability of these fungi to synthesize the aflatoxin depends on suitability of crop genotype for its climate, insect damage, relative humidity, agricultural practices as well as "postharvest" conditions such transportation, storage, and food processing (Wu and Khlangwiset, 2010). Maize and peanuts are highly consumed in the world and are therefore the main sources of human exposure to aflatoxin (Lizárraga-Paulín, 2011).

When aflatoxins are consumed, it can cause toxicity by interfering with the integrity of the intestine or control the expression of cytokines and can cause abnormal growth in the immunesuppressed individuals and/ infants. Aflatoxins may be transformed in the liver by certain p450 enzyme to form Aflatoxin-8-9-epoxide which binds to liver proteins and lead to their failure, it then results in acute aflatoxicosis or it may bind to DNA, contributing to aflatoxin induced hepatocellular carcinoma (liver cancer) (Chibuike, 2015).

These effects have not been extensively studied in humans, but there are evidence suggesting that at least some of the effects observed in animals also occur in humans. About 4.5 billion persons living in developing countries are chronically exposed to uncontrolled amounts of the toxin to a large extent (Williams *et al.*, 2004) of which Ghana is of no exception. A research conducted by Kumi *et al.* (2014) on Aflatoxins and Fumonisins contamination of home-made weanimix; an infant weaning food made from roasted milled corn fortified with legume revealed that children in Ejura Sekyedumase District in the Ashanti Region of Ghana who were fed on weanimix were exposed to high levels of aflatoxin and Fumonisins.

2.1.1-5 Treatment of aflatoxicosis

Novasil clay, a Calcium montmorillonite clays an anticaking agent has been demonstrated to detoxify aflatoxin in animals when ingested. The result of a research conducted by Philips *et al.* (2008) showed that, it binds to aflatoxins with high affinity and high capacity in the gastrointestinal tract, resulting in a notable reduction in the bioavailability of these toxins without interfering with the utilization of vitamins and other micronutrients. A study by Afriyie-Gyawu *et al.* (2008) in Ghanaians at high risk for aflatoxicosis has indicated that when Novasil Clay in a capsule form, is administered orally at dose level of 0.25 %, it decreases biomarkers of aflatoxin exposure and does not interfere with the levels of serum vitamins A and E, and iron and zinc. In an environment where the risk of aflatoxin exposure is high, the administration of Novasil clay orally is recommended for the protection of humans against their harmful effect. However, there also exist the possibility of treating aflatoxin B_1 with ammonium hydroxide. Invitro experiment reveals that, ammonium hydroxide will react with aflatoxin B_1 at room temperature yielding a brown product that is nontoxic in a chick embryo bioassay (Vesonder *et al*., 1975).

2.1.2 Ochratoxins

These are also naturally occuring mycotoxins produced by certain species of *Aspergillus* and *Penicillium* (*P. verrucosum* and *P. carbonarius*). Ochratoxin is made of three secondary metabolites from which are ochratoxin A (OTA), ochratoxin B (OTB) and ochratoxin C (OTC). Ochratoxin B (OTB) is a form of ochratoxin A (OTA) that is nonchlorinated. Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A (Bayman and Baker, 2006). OTA, according to Mateo *et al.* (2007) is a possible carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract. OTA is most frequent in stored grain. There are school of thoughts that OTA was linked to kidney disease and damage to DNA (Heather and Snedeker, 2004).

2.1.3 Citrinin

It is a toxin that was first isolated from *Penicillium citrinum* and is lemon-yellow in colour. It is less toxic compared to aflatoxin and ochratoxin. It can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys (Bennett and Klich, 2003). However, contrary to aflatoxin, a publication of EFSA Journal in 2012 revealed that citrinin is heat sensitive and decomposes to form other complex compounds such as Citrinin H_1 and H_2 which have higher and weaker cytotoxicity respectively.

2.1.4 Ergot

They are toxic mixture of alkaloids produced by species of fungi (*Claviceps purpurea*) contaminate rye grains. A person suffers from ergotism historically known as St. Anthony's Fire when he or she eats bread or any food item made from contaminated rye. This disease is characterized by convulsion, defect in central nervous system (Bennett and Klich, 2003)

2.1.5 Patulin

They are mycotoxins produced by the *Aspergillus species*, *Penicillium expansum*, and *Paecilomyces species*. Patulin is mostly associated with moldy fruits and vegetables, in particular rotting apples and figs (Moss, 2008; Trucksess and Scott, 2008). It is destroyed during fermentation process and so is not found in apple beverages, such as cider.

Patulin affects the immune system even though it is not carcinogenic (Moss, 2008). The maximum acceptable limit as set by European Community in 2004 is 50 µg/kg in all fruit juice concentrations, at 25 µg/kg in solid apple products for consumption, and at 10 µg/kg for apple products meant for children, including apple juice (Moss, 2008; Trucksess and Scott, 2008).

2.2 STATISTIC ON THE PRODUCTION AND CONSUMPTION OF CEREALS IN GHANA

Maize and rice are among the products frequently consumed by Ghanaians. The domestic demand for both increases yearly. Between 2010 and 2015, rice demand was projected to grow at a compound annual growth of 11.8 % and maize at 2.6 % resulting in a short fall of domestic supply of 12 % for maize (Armah, 2010).

The Analysis of maize consumption data in Ghana by Boateng *et al.* (1990) revealed that maize and maize products contributed to 10.8 % of food expenditure of the poor and 10.3 % of food expenditure of all income groups.

2.3 THE SIGNIFICANCE OF THE DISC ATTRITION MILL (CORN MILL OR 'NIKA NIKA') IN THE GHANAIAN COMMUNITY

In the Ghanaian community, Disc Attrition Mills is known as the Corn mill but popularly called 'Nika Nika' (Achiaa, 2015). Their services have been and are still being patronized by almost every household. It therefore forms a major part of our food processing chain. The services are not only patronized by individual households, but also small scale food manufacturers. This category of manufacturers who patronize their services as reported by FDA (2014) forms about 70 % of small -to- medium scale manufacturers. This is because they lack the necessary capital to acquire a milling machine for the purposes of their production and therefore rely on the services of these corn mills. This percentage is so huge that their contribution to food safety cannot be overlooked. It is therefore of paramount concern, the hygienic condition of these facilities.

The corn mill also plays an important role in the preparation of most Ghanaian dishes. It is used to mill cereals (corn, sorghum, millet, rice, barley etc), roots and tubers (cassava etc), legumes (beans, peanuts), vegetables (pepper, tomatoes), nuts (palm kernel). These ingredients are used to prepare the following Ghanaian dishes; banku, kenkey, kokonte, tuo zaafi, koko, tubani, tom brown and weanimix. Groundnut soup, enjoyed by most Ghanaian starts here. The groundnuts are processed into paste and subsequently the soup. It can therefore be described as the friend of the food vendor, in Ghana.

2.3.1 Contribution of the disc attrition mill to food safety

Food safety is the assurance that food will not cause harm when prepared and/or eaten according to its intended use (Codex Stan, 1969).

In as much as the disc attrition mills are of immense benefit to the Ghanaian populace as well as the small to medium scale manufacturers, the commercial mill also poses serious threat to food safety and thus public health. The premises of most of these facilities are in unhygienic conditions. Essel *et al*. (2015) reported that, 21 % of disc attrition mills audited in the Accra metropolis failed good manufacturing practices (GMP), 77 % were operating under very poor hygiene conditions and 2 % were operating under appreciable level of hygienic conditions.

The activities in this sector are either minimally regulated or not regulated at all so there are possibilities of introducing hazards into the products to be milled. As part of routine maintenance the machine parts including the hopper are painted. The paints peel off and eventually go into the food. Most of the paints are lead based (Howell, 1998). Lead can damage the central nervous system, cardiovascular system, reproductive system, hematological system, and kidney.

During milling, iron filings may contaminate the food from the grinding disc and the level of contamination increases with the quantity of milled product. However, when the product is to be milled 'dry', the rate of metal loss decreases at first and then increases with mass of milled product, where as in 'wet' milling, the rate increases monotonically with mass of milled product (Kwofie *et al.,* 2011). The difference could be explained in terms of the mechanism(s) or mode(s) of metal loss under the different milling conditions. In 'dry' milling, loss of metal is initially controlled by friction wear and later by erosion-corrosion. In the case of 'wet' milling metal loss could be attributed to conjoint action of corrosion and friction wear. This poses possible health effects on consumers of foods prepared from such contaminated milled product.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIAL

Maize samples were purchased at Anloga market, Kumasi, Ashanti Region at each sampling point. Acetonitrile, sodium chloride, potassium bromide, nitric acid, methanol and aflatoxin standard stock solutions were purchased from Sigma Aldrich, Germany. The analysis was done at the mycotoxin laboratory facility at Kwame Nkrumah University of Science and Technology using high performance liquid chromatography (HPLC).

3.2 STUDY AREA

The study was conducted in Kumasi. It is the capital city of the Ashanti Region of Ghana. The choice of Kumasi as the study area was due to the fact that it is the most populated city in Ghana even more populated than Accra, the nation's capital city. Its proximity to the aflatoxin laboratory at KNUST, where samples for this work were analyzed was also a factor. The Kumasi metropolis is about 254 sq/km and has a population of 2,035,064 as against Accra metropolis of 1,848,614 (Ghana Statistical Service, 2010). The population of Kumasi metropolis has an annual growth rate of 4.8 % but that of Accra metropolis is 3.1 %. Kumasi is located at the transitional forest zone. It is about 270 km north of the nation's capital, Accra and lies between latitudes 6.35^{o} and 6.40^{o} as well as longitudes 1.30^{o} and 1.35^{o}. It is about 300 m above sea level (Kumasi Metropolitan Assembly, 2013).

Kumasi metropolis has about ten (10) sub-metros which are Asokwa, Kwadaso, Nhyiaeso, Bantama, Tafo, Asawase (Zabozongo, Aboabo), Manhyia (Tafo Pankorono, New zongo), Suame, Oforikrom and Subin. Please find below in figure 2, the map of the sub-metro areas in Kumasi

prepared by town and country planning department, Kumasi in 2008. The white arrow indicates the sub metro selected from those dominated by residents of low income earners (Subin) while the black arrow indicate the sub metro selected from those dominated by residents of high income earners (Asokwa).

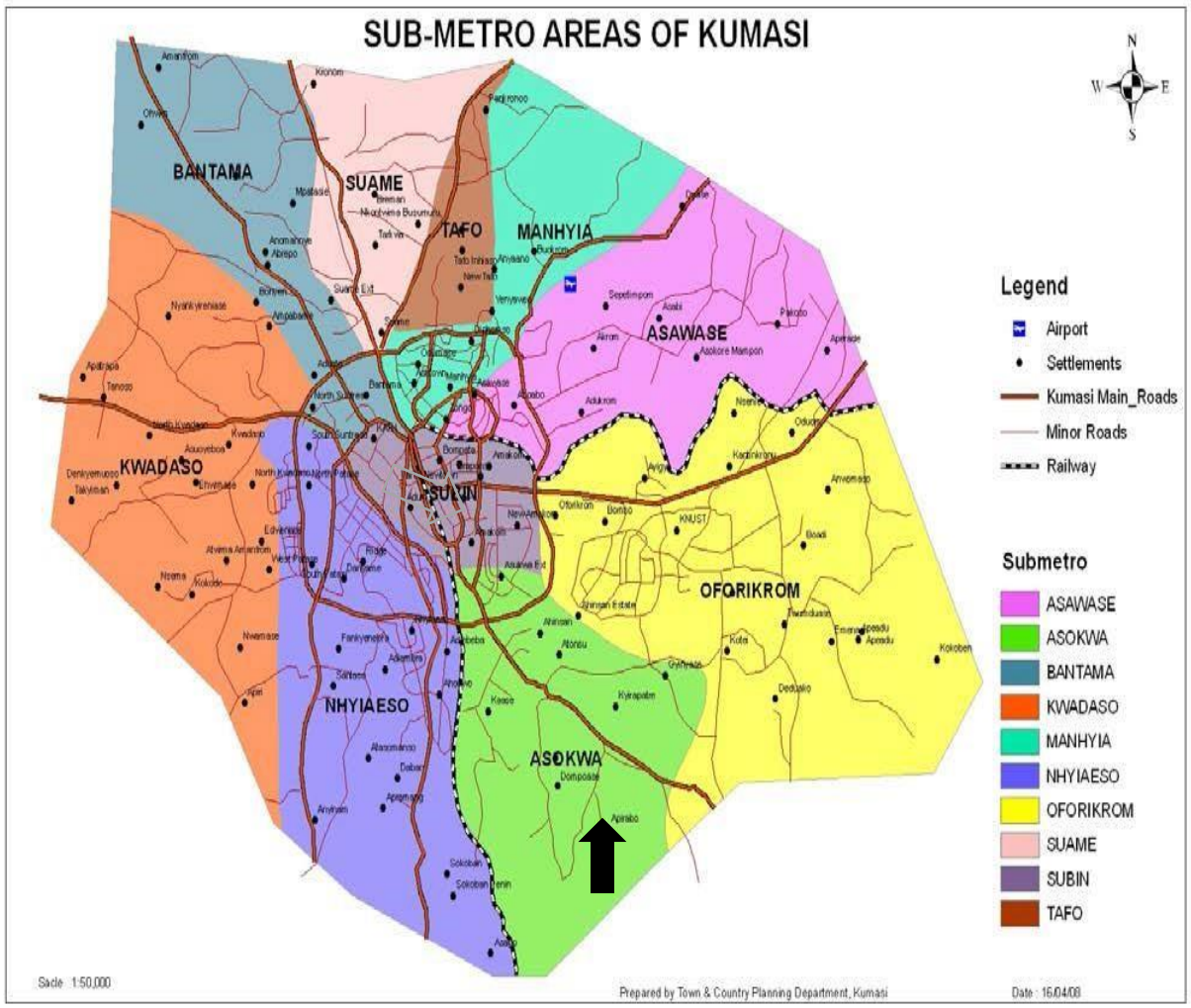

Figure 2: The map of Kumasi Metropolis Source: Nendomachuli (2011)

3.3 METHODS

3.3.1 Selection of disc attrition mills for the study

The stratified sampling method was adopted in selecting the commercial milling facilities for this study. The ten (10) sub-metros under the Kumasi metropolis were grouped into two (2) subgroups through a mini survey conducted where residents that were familiar with Kumasi metropolis were asked to classify the ten (10) sub-metros into two (2) socio-economic subgroups. Each subgroup indicated a stratum with similar socioeconomic (social, economic and educational) status.

Stratum A, comprised sub-metros that were revealed by the survey to be dominated by residents that are high income earners (H). They were residents of higher social, economic and educational status. These were Asokwa, Kwadaso, Nhyiaeso, Bantama and Tafo. Among them, one (1) submetro (Asokwa) was selected at random using the randomizer in excel. Two (2) corn mills were selected from this submetro based on their willingness to co-operate. They were located at Kaase (H_1) and Mayanka (H_2).

Stratum B, comprised of sub-metros that were revealed by the survey to be dominated by residents that are low income earners (L). They were residents of lower social, economic and educational status. These sub-metros were Subin, Asawase (Zabozongo, Aboabo), Manhyia (Tafo Pankorono, New zongo), Suame and Oforikrom. Among them, one (1) sub-metro (Subin) was selected at random using the randomizer in excel. Two (2) corn mills were selected from this submetro based on their willingness to co-operate. They were located at Amakom (L_1) and near the Apostolic church (L_2).

A total of four (4) commercial milling facilities were selected and used for the study. Two (2) located in Asokwa (H) and two (2) located in Subin (L).

3.3.2 Training of officers and operators of the disc attrition mill

An officer was trained on how to audit the commercial milling facility against the Good Manufacturing Practices (GMP) checklist developed. They were also trained on how to store and transport the samples to the various commercial milling facilities. The operators of the disc attrition mills were also educated on the purpose of this project and were assured of their confidentiality and so their consents were sought before using their facilities. Anloga market women from whom the maize samples were purchased were also sensitized about the inconvenience the project was likely to bring them and the fact that they had to be available at any point that their services were needed.

3.3.3 Administration of semi structured questionnaire (GMP Checklist)

The selected disc attrition mills were audited against a modified ICD-UGL 2013 Good Manufacturing Practices Checklist (Appendix 1 in page 55 shows the detailed check list for the four commercial milling facility of which they were audited against). The intent of the audit was explained to the operators of the selected disc attrition mills. The GMP audit was to ascertain the knowledge of the operators in quality assurance so as to guarantee the safety of the product milled, to assess the facility design and layout describing the structure and fabrication, to ascertain whether the location of the facility is suitable for its purpose, to ascertain if the facility is regularly maintained, to ascertain if the equipment are designed in a manner that is not a threat to food safety and finally to establish if there is a corrective action for an identified critical control point during operation. These were realized by physical observation, interaction with the operators and review of relevant available document. Photographs were also taken to emphasize on the results obtained with permission of the operators.

The operators were also asked for the day their sales were maximum (Max) as well as minimum (Min). These days were noted to be Wednesdays and Thursdays respectively. The time within which a lot of clients patronized the facility (peak period) was in the afternoon. The samples were sent to all the facilities in the morning and peak period on both days.

3.3.4 Preparation of maize sample for milling

Maize samples were purchased from the market at each sampling point and filled into Low Density Polyethylene (LDPE) zip-lock bags. These were wrapped with aluminium foil to prevent the destructive effect of ultra violet light and florescent on aflatoxin, appropriately labeled for easy identification (see appendix 2 in page 79 for the labeling codes), stored and transported to the commercial milling facility in an ice chest filled with ice so as to stop or minimize the activities of the moulds as well as the production of aflatoxin.

3.3.5 Distribution of maize sample to the corn mill facility

Day of maximum sale (Max) - Wednesday Morning (Morn)

In the morning of the day of maximum sale, five (5) Low Density Polyethylene (LDPE) zip-lock bags (size 10), each containing, 2 kg of maize samples purchased from the market and were wrapped with aluminium foil and appropriately labeled (L_1-Max-Morn, L_2-Max-Morn, H_1-MaxMorn, H_2-Max-Morn, C-Max-Morn) for proper identification. The four (4) were immediately dispatched to their respective mills in an ice chest filled with ice. They were the first products to be milled that morning. The remaining one (1) which served as a control (C) and accounted for aflatoxins accumulated during harvesting, transportation, distribution and storage till that time was blended in the laboratory. The milled samples were immediately returned to the laboratory for analysis in the same storage conditions.

Plate 1: Image showing the milling process

Peak period (Pk)

At the peak period of the day of maximum sale, a different set of five (5) Low Density Polyethylene (LDPE) zip-lock sachets (size 10), each containing, 2 kg of maize samples purchased from the market were wrapped with aluminium foil and appropriately labeled (L_1Max-Pk, L_2-Max-Pk, H_1-Max-Pk, H_2-Max-Pk, C-Max-Pk) for proper identification. The four (4) were immediately dispatched to their respective mills in an ice chest filled with ice. They were milled after a number of clients have been attended to during the day. The remaining one (1) which served as a control

(C) was blended in the laboratory. The milled samples were immediately returned to the laboratory for analysis in the same storage conditions.

Day of minimum sale (Min) - Thursday Morning (Morn)

In the morning of the day of minimum sale, another set of five (5) Low Density Polyethylene (LDPE) zip-lock bags (size 10), each containing, 2 kg of maize samples purchased from the market were wrapped with aluminium foil and appropriately labeled (L_1-Min-Morn, L_2-MinMorn, H_1-Min-Morn, H_2-Min-Morn, C-Min-Morn) for proper identification. The four (4) were immediately dispatched to their respective mills in an ice chest filled with ice. They were the first products to be milled that morning. The remaining one (1) which served as a control (C) and accounted for aflatoxins accumulated during harvesting, transportation, distribution and storage till that time was blended in the laboratory. The milled samples were immediately returned to the laboratory for analysis in the same storage conditions.

Peak period (Pk)

At the peak period of the day of minimum sale, a final set of five (5) Low Density Polyethylene (LDPE) zip-lock bags (size 10), each containing, 2 kg of maize samples purchased from the market were wrapped with aluminium foil and appropriately labeled (L_1-Min-Pk, L_2-Min-Pk, H_1-Min-Pk, H_2-Min-Pk, C-Min-Pk) for proper identification. The four (4) were immediately dispatched to their respective mills in an ice chest filled with ice. They were milled after a number of clients have been attended. The remaining one (1) which served as a control (C) was blended in the laboratory. The milled samples were immediately returned to the laboratory for analysis in the same storage conditions.

3.3.6 Laboratory analysis of the samples for aflatoxin

3.3.6-1 Safety Requirement

The raw maize and the milled maize were handled with caution with the suspicion of being contaminated with aflatoxin. Protective gloves and nose mask were won during grinding since it emits dust which might carry or contain traces of aflatoxins. The work on the sample was done in the fume hood because they were possible carcinogens. Accidental spill of the toxin were swabbed with 1 % sodium hypochlorite bleach (NaOCl), left for 10 min and then 5 % aqueous acetone was added. All equipment and glass wares suspected to be exposed to aflatoxin were rinsed with methanol with the addition of 1 % sodium hypochlorite solution (NaOCl) and 5 % acetone was added and allowed to react for 30 min. The equipment and glass wares were then washed thoroughly.

Laboratory coat or apron suspected to be exposed to aflatoxin were soaked in 5 % Sodium Hypochlorite solution (NaOCl) and left overnight. It was then washed in water and dried before use.

Analytical materials were adequately protected from daylight and aflatoxin standard solutions were also kept protected from light by using amber vials covered with aluminium foil.

The use of non-acidic washed glass ware aflatoxin aqueous solution may cause loss of aflatoxin. So instead, a new glass ware was soaked in dilute acid for several hr, and then rinsed extensively with distilled water to remove all traces of acid before use (AOAC, 2000a).

3.3.6-2 Sample preparation

The raw maize was homogenized using a high speed blender, passed through a no. 20 sieve and mixed thoroughly before extraction. This is to form a uniform mixture because, aflatoxin contamination in grains was likely to be in pockets and not uniformly distributed (AOAC, 2000b).

3.3.6-3 Preparation of standard for aflatoxin analysis

Aflatoxin standards (ng/g) were prepared from stock solution of Supelco aflatoxin B_1, B_2, G_1 and G_2 of 2.6 ng/µL in methanol by diluting them to a concentration of 0.5 µg/mL.

3.3.6-4 Preparation and storage of working standards

Before storage the flask was weighed to the nearest mg and the weight recorded for future reference. The flask was wrapped tightly in aluminium foil and stored at 0 ^{o}C.

To avoid incorporation of water by condensation, all standards were brought to room temperature before use. The purity of the standard was checked by determining chromatographic purity and molar absorption and the concentration calculated (AOAC, 2000d).

3.3.6-5 Fluorescence detector conditions

Detector parameters which were applicable for the available equipment was used according to the manufacturer's recommendations. The optimum condition for the fluorescence detector were Excitation, 360 nm; emission, 435 nm; filter mode, resistor-capacitor circuit (RC) response setting of slow; digital filter, 3 or 5 s; gain, 10 or above; attenuation, 1 (AOAC, 2006).

3.3.6-6 Drawing the standard curve

To establish a standard curve, five (5) concentrations of a mixed aflatoxin standard containing from 0.1 to 1.0 ng toxin per 20 mL injection solvent were prepared. These individual extracts were evaporated to dryness under nitrogen in silanized vials. Subsequently, each of the extracts with 2.0 mL injection solvent were reconstituted and stirred with a Vortex mixer for at least 2 min.

3.3.6-7 Principle of HPLC

Aflatoxins were extracted using sodium chloride and methanol and purified using vicam aflatest immunoaffinity column (IAC), derivatised with tri flouroacetic acid (Aflatoxin B_1 and G_1 to B_2 to G_2 respectively), separated by reverse phase liquid chromatography and detected by fluorescence. The method measured up to 0.1 ng of aflatoxin B_1, B_2, G_1, G_2. Detection limit was 0.5 ng/g.

3.3.6-8 Apparatus

Liquid chromatograph: Rheodyne Septumless Injector, Flourichrom Fluorescence detector, excitation filters, glass emission filters fitted with flow integrator or recorder, chart speed.

LC column: Supercoisil LC. New LC column or those that have been stored in methanol for extended period require conditioning with concentrated standard in order to achieve maximum resolution and sensitivity to aflatoxin B_1 and G_1.

Cleanup column: Teflon stopcock and coarse frit bed support, detachable glass solvent reservoir.

Others: Adjustable pipette with disposable tips, Filter tubes with coarse frit bed support, high speed blender-explosion proof (8000 rpm), Vertical shaker-adjustable (for maximum solid liquid agitation).

3.3.6-9 Reagents

Solvent-distilled-in-glass grade methanol, hexane, methyl chloride, benzene, acetone, acetonitrile, anhydrous ethyl ether stored in metallic container. (-glass ether bottled form peroxide soon after opening which degrades aflatoxin)

LC elution solvent H_20-CH_3CN-CH_3OH (700 + 170 + 170). The water was adjusted to obtain baseline resolution of aflatoxin B_2 and G_2

Silica gel for column chromatography-Silica gel 60 (0.063 - 0.2 mm). Activated by drying at 100 ^oC. Cooled to room temperature. Desired quantity (100 g) was weighed into a glass stoppered container. Water (1 mL) was added in small increment. Silica gel was agitated between additions. It was then shaken mechanically 4-6 hr and left to stand for 16 hr.

Triflouroacetic acid (TFA)-Assay by titration equal to or more than 95 %. About 1-2 mL of TFA was transferred to a 1 dram vial with Teflon lined cap. The vials were kept in freezer when not in use and discarded if discoloration occurs.

Sodium sulphate anhydrous: Fines were sifted out to obtain 20-40 mesh and heated for 2-3 hr at 600 ^oC to remove organic impurities.

Aflatoxin standard solution:
Aflatoxin stock solution - individual stock solutions were prepared using benzene-CH_3CN (98 + 2) and the concentration of each was determined by measuring UV absorption.

Working standard solution - Eppendorf pipette was used to transfer appropriate quantity of stock Solution to each 4 dram vial to obtain final concentrations of aflatoxin in each vial as indicted in Table 2 below:

Table 2: Amount of aflatoxin in stock solution of each 4 dram vial

Vial	**B_1 and G_1 ng**	**B_2 and G_2 ng**
1	250	125
2	500	250

3	1000	500
4	2000	1000

The solution was evaporated to dryness under gentle steam of nitrogen. Using Eppendorf pipette, 200 µL hexane and 50 µL TFA was added to each vial cap and vortex mixed for 30 min. The solution was left to stand for some time and H_2O-CH_3CN (9 + 1) was added and then vortex mixed. The solution was centrifuged and the layers separated to obtain the final concentration as indicated in Table 3.

Table 3: Final concentration of aflatoxin

vial	B_1 and G_1 ng/10.05 mL	B_2 and G_2 ng/10.05 mL
1	0.250	0.125
2	0.500	0.250
3	1.000	0.500
4	2.000	1.000

3.3.6-10 Extraction and Clean-up

A mixture of ground sample (25 g) with 5 g of sodium chloride and 125 mL of methanol/deionized water (70:30) was blended at high speed for 2 min and filtered through a fluted filter paper. The extract (15 mL) was diluted with 30 mL of deionized water and filtered through a 1.0 µm microfiber filter. The diluted extract (15 mL) was passed through Vicam aflatest immunoaffinity column (IAC) with 1 mL HPLC grade methanol and 100 µL of eluent was injected into the HPLC. Plates 10 and 11 in appendix 3 in page 80 show photographs of the extraction procedure.

3.3.6-11 HPLC Determination

Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360 nm, Em: 435) with Phenomenex Hyper Clone BDS C18 Column (150 x 4.60 mm, 5 µm) were used for aflatoxin detection and quantification. The mobile phase that comprised of isocratic (60:10:30) of (water:ACN:MeOH) with 119 mg of potassium bromide and 350 µL of 4 M nitric acid (HNO_3) was required for post column electrochemical derivatisation with Kobra Cell (100 µA), R-Biopharm Rhone at a flow rate of 1 mL/min. Calibration curve of aflatoxin mix (B_1, B_2, G_1 and G_2) was constructed by injecting 25 µL of derivatised standard solution of aflatoxins (B_1, B_2, G_1 and G_2. The curve was used to check linearity of response using the standard. Limit for detection and limit for quantification for total aflatoxin was established at 0.5 ng/g and 1 ng/g respectively.

3.4 DATA ANALYSIS

The data from the HPLC analysis on aflatoxin levels were subjected to a one sample 'T' and a two sample 'T' distribution at a 95 % confidence level using a minitab-Statistical Package 16 developed by Minitab inc, State College, Pennsylvania, U.S.A.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 GENERAL GMP ASSESSMENT

In general, the performance of the four commercial milling facilities against Good Manufacturing Practices was not satisfactory. On average, the four commercial milling facilities audited scored below 25 % which means either there was no reasonable evidence that the GMP check item was adequately addressed or there existed only little amount of evidence that the GMP check item was being addressed and the efforts were not enough to eliminate or minimize the threat to public health and safety.

4.1.1 General Control

This section of the checklist sought to find out information on Personnel management. The check items under this section revealed the level of knowledge the operators had in food safety and quality assurance. The observation, documentary evidence and response given by the operators revealed that they had no knowledge in good hygiene practices. The facilities on the average scored below 25 % under this section which is not encouraging. On the average, each facility had 2 operators of whom none were the owners. Some owners visited their facilities at the close of work to collect the money made for the day. Others seldomly visit the facility. For these, the money made for the day were kept and sent to them by the operators on weekly basis. Perhaps the owners were much more interested in the revenue that was generated than the day to day activities.

4.1.2 Equipment Control

This included the placement of the equipment and its maintenance. All the four facilities audited scored 25 % which means that despite their efforts in maintaining the equipment in good shape, more needs to be done. There were no maintenance regimes in place. The equipment parts were changed or repaired only when they were completely out of use (shut down for maintenance) and thereby affecting revenue generation.

4.1.3 Materials and Operational Control

In terms of quality control of the raw material, the performance was below 25 %. The quality of the raw material was not assured by the operators before milling. This may have had varying consequences on products of clients that may be of good quality initially.

4.2 SITING OF THE DISC ATTRITION MILLS (CORN MILL)

In the choice of a suitable location for a food processing facility, the influence of the activities of the adjoining facilities and the external environment on food safety must consider be considered.

The facilities were also evaluated based on the suitability of their location and the maintenance culture put in place. They were scored below 25 % on the average. The facilities require improvement under this category. The audit report revealed that due diligence was not done in choosing the site for establishing the facilities. The location of some of the facilities was inappropriate. The facilities were prone to dust, fumes and vehicular activities. The activities of the adjoining facilities greatly interfered with their smooth operation. One of the facilities was sited in an environment allocated for rearing and selling livestock and was also not far from a public toilet that was in bad state. The stench emanating from the animal droppings had great impact on the smooth running of the facility.

Plate 2: The facility located at an evironment where animals are reared

Maybe as per the owner's point of view, the prima facie requirement for establishing a corn milling facility was its non-availability in a particular location. Once this requirement was not met, it was then established ignoring the environmental factors and the influence of the adjoining facilities. This result is comparable to the work done by Essel *et al.* (2015) on the Contribution of Commercial Mills to Food Safety in Ghana.

4.3 FACILITY CONSTRUCTION CONSIDERATION

An ideal food processing facility must be constructed such that, it provides protection for food to be processed, the processing facility and the processed product from contamination. However, the four corn milling facilities visited did not meet this requirement. The facilities were not constructed in a manner to restrict the entry of pest and other domestic animals and they had no documentary evidence of pest management. Some of the facilities audited had animals such as goats, fowls, birds and rats that freely moved in and out of them (Plate 3).

Plate 3: Some domestic animals seen coming in and out of the facilities

Some of the facilities audited had their walls elevated from the foundation level with concrete material and continued with wood. Others were completely constructed with wood (see Plate 4 in page 35).

Plate 4: Some of the materials used in constructing the facilities

The facilities were roofed with galvanized aluminium sheets and had no ceiling. The roofing had exposed wires and thereby put the operators at high risk of electrocution (Plate 5 below).

Plate 5: Galvanized aluminium sheet used in roofing without ceiling

The floors were made of concrete but had deteriorated with time (floors had cracks and holes) due to lack of maintenance.

4.4 SANITATION

The facilities had no documented standard operating procedure for cleaning and disinfection. Cleaning was done as and when the operators deemed it necessary. Short hard brooms and dirty rags were used in cleaning the equipment as well as removing remnants from the grinding discs.

Plate 6: Some cleaning devices used in tidying up the facilitiy

The drains were choked with garbage. The ceilings and walls were covered with cobweb and the floors were occupied with redundant items such as equipment parts, empty sacs etc. that could harbor rodents.

Plate 7: Some redundant items kept in the facilities

The operators had no dining rooms. Cooking and dining were done at the same premises.

Plate 8: Cooking done in the same facility

The hopper and some other equipment were not continuously welded together and thereby created dead areas which were not easily accessible during cleaning. Food particles continuously got stuck in these areas and eventually formed biofilms. This poses threat to food safety.

Plate 9: Discontinuous welding of the hopper creating dead areas

The grinding discs were dismantled and cleaned only if they were about to start the day's activities or if the subsequent product to be milled was totally different from the preceding one. Bhadriraju (2013) also raised sanitation issues that confronted a flour mill in Ho Chi Minh, Vietnam. These included inadequate pest control regime, poor maintenance culture, and open drainage system filled with garbage. These issues are comparable to those revealed by the audit of this current work.

4.5 PERSONNEL MANAGEMENT

The highest level of education of the operators was Senior Secondary School Certificate. They had no protective clothing and no insurance cover in case of any occupational hazard. They had no training in quality assurance and had little knowledge of food safety.

4.6 EXTENT OF AFLATOXIN CONTAMINATION IN MAIZE SOLD IN THE MARKET

The data from the controls (represented aflatoxin level of maize sold in the market) were compared to the maximum acceptable limit of aflatoxin in maize in Ghana (20 ppb) as stipulated in Ghana Standard (2003). A one sample 'T' distribution was used at a 95 % confident level. With a p-value < 0.05 (significance value), a standard deviation of 4.72 and a mean of the controls of 70 ppb, the levels significantly exceeded the maximum acceptable limit of more than three folds. This posed a health risk to the consumers.

It was revealed in a study by Akrobortu (2008) on the contamination of maize from different storage locations in Ghana that, the level of aflatoxin in grains was likely to increase in locations with high humidity and rainfall pattern. Kumasi is also located around the middle belt of Ghana and as according to Abubakari (2013) has recorded consistently high rainfall over the past 20 years with relatively high humidity. This reason, coupled with improper storage might have accounted for the significantly higher aflatoxin levels of maize sold in the market. In the same study, districts with the highest rainfall and humidity had the highest aflatoxin levels in maize over a ten year period. The current research is in support of the findings of Hell (1997), who also researched into the aflatoxin contamination of maize in zones in Benin and concluded that zones with higher relative humidity tested higher for aflatoxin levels. Kaaya and Warren (2005) revealed that, storing maize above fire racks was the only known method to protect the maize against aflatoxin contamination. But unfortunately, the method was limited to only smaller quantity of grains.

There is indeed the need for improvement in the post-harvest handling conditions of the maize so as to prevent or minimize the synthesis of the aflatoxin by moulds.

4.7 LEVELS OF CHANGE OF AFLATOXIN IN THE TEST SAMPLE AT EACH SAMPLING POINT Day of maximum sale (Wednesday) Morning

The data from the four (4) samples distributed to the disc attrition mills in the morning of the day of maximum sale were analyzed as against the control (74.05 ppb) using a one sample 'T' test. A p-value < 0.05 (significance value), a standard deviation of 7.34 and a mean of 95.8 ppb indicated a significant increase in aflatoxin levels in the samples milled with a percentage increase of 29.3. This increment may be attributed to the fact that, after the previous day's work, the residues/remnant that got stuck in the grinding discs were either partially removed or not removed at all. And there also existed conditions favourable for mould growth and thus aflatoxin production. This resulted in the high levels of aflatoxin in the test sample.

Peak period

The data from the four (4) samples distributed to the disc mills at the peak of the day of maximum sale were analyzed as against the control (74.26 ppb) using a one sample 'T' distribution. A p-value < 0.05 (significance value), a standard deviation of 14.06 and a mean of 107.32 ppb also indicated that, the increase in aflatoxin levels was significant and it had a percentage increase of 44.5. This may also be attributed to the fact that, the previously milled products may have been pre-contaminated with aflatoxin which in effect contaminated the test sample using the disc attrition mills as a medium of contact. The basic condition of ensuring food safety at high levels is to supply low risk raw materials (Erbas *et al*., 2013). High risk raw materials run through the disc attrition mills and thus increasing the aflatoxin level of the test sample.

Day of minimum sale (Thursday) Morning

The data from the four (4) samples distributed to the disc attrition mills in the morning of the day of minimum sale were analyzed against the control (65.77 ppb) using a one sample 'T' test. A

mean of 96.6 ppb showed a percentage increase of 46.9. This indicated a change in the mean aflatoxin levels of the test sample. However, a p-value > 0.05 (significance value) showed that, the change was not significant. A standard deviation of 21.7 implied that the values in the data set were farther from the mean (96.6 ppb) justifying the huge percentage increase of the mean aflatoxin levels of the test samples.

Peak period

The data from the four (4) samples distributed to the disc attrition mills at the peak of the day of minimum sale and the control (66.19 ppb) were analyzed. A mean of 100.6 ppb showed a percentage increase of 51.9. This also indicated a change in the mean aflatoxin levels of the test sample. However, a p-value > 0.05 (significance value) showed that, the change was not significant. A standard deviation of 38.9 also implied that the values in the data set were farther from the mean (100.6 ppb) justifying the huge percentage increase of the mean aflatoxin levels of the test samples.

The insignificant change in aflatoxin levels of the test samples distributed to the disc attrition mills in the morning and peak period of the day of minimum sale could be explained that, only few clients patronzed the facility and the probability of the test sample getting contaminated was low.

4.8 AFLATOXIN LEVELS IN THE TWO SOCIOECONOMIC GROUPS (LOW INCOME EARNERS / HIGH INCOME EARNERS)

The data from the two socioeconomic groups were compared using a two sample 'T' distribution at a 95 % confidence level. Comparing the mean values of data from lower income earners communities (110.9 ppb) and high income earners communities (89.3 ppb), there was a mean difference of 21.14 ppb in the aflatoxin levels of the two socioeconomic groups. With a p-value <

0.05**,** the difference was significant. Figure 3 below gave details about aflatoxin levels in the two socioeconomic groups.

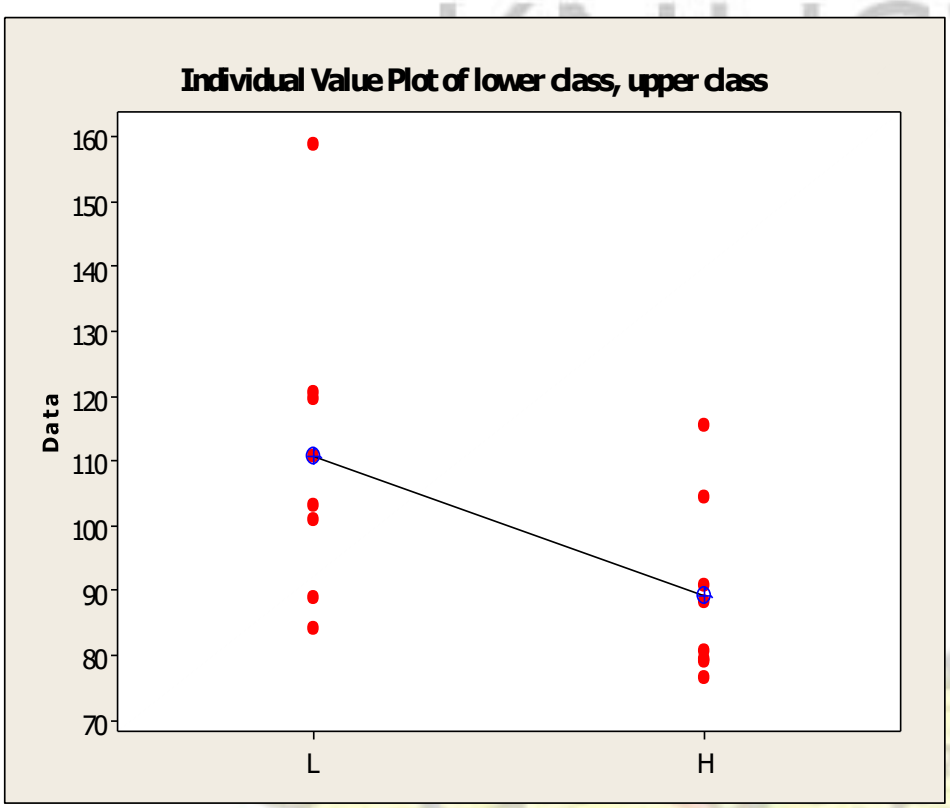

Figure 3: The mean aflatoxin values of the two socioeconomic groups

It could be deduced from the graph that, test samples from the lower class communities increased in the aflatoxin level far more than those from the upper class with mean values of 110.9 and 89.3 respectively. The increased levels of aflatoxin in the test sample from the lower class community could be explained that, majority of the residents in these communities could only afford low grade maize for their various dishes. And this low quality maize which are often processed by the disc attrition mills in the same communties may have been contaminated with aflatoxin and in effect increased the aflatoxin levels of the test samples. Test samples from the urban communities also had significant increase in aflatoxin level because aflatoxins were almost inevitable despite the

preventive measures that might be put in place. It could also be due to longer storage period of grains (Hell *et al.*, 2000) in their various homes under favourable conditions of humidity and temperature necessary for mould growth and thus contaminating the test sample using the disc attrition mills as a medium of contact. The socioeconomic condition of a particular locality therefore had influence on the aflatoxin levels of products milled by the corn mill in that locality.

The current research supports a study done by Ahsan *et al.* (2010) on aflatoxin levels of maize grains and maize flour in both rural and urban communities of Faisalabad division of Pakistan. The highest prevalence rate of fungi that produce aflatoxin were found in samples from the communities of lower class compared to the upper class.

The study of Chelule *et al.* (2001) on exposure of rural and urban populations in KwaZulu Natal, South Africa, to fumonisin B_1 in maize affirmed that the rural folks were exposed to higher levels of mycotoxins than the urban.

CHAPTER FIVE

CONCLUSION

The GMP audit revealed that, the disc attrition mills were operated under unhygienic condition and that there were sanitation issues to be addressed.

Results obtained from the laboratory as well as the analysis of data also revealed that the disc attrition mills was a medium for aflatoxin contamination of maize flour and that the level of contamination was dependent on the hygiene situation at the facility, the socioeconomic status of the people living in the community in which the facility is located and the quantity and quality of grains that are processed by the corn mill.

RECOMMENDATION

As a short term measure, clients are advised to run a little more of their samples through the disc attrition mills thoroughly before the actual sample so as to reduce remnants that might have gotten stuck in the grinding discs. This will reduce the risk of contamination of the actual products.

However, for a long term measure, policy makers, regulatory authorities, District, Municipal and Metropolitan assemblies under the ministry of local government and rural development and all other relevant institutions should come out with policies and guidelines to streamline and regulate the activities of this sector.

REFERENCES

Abbas, H., Zablotowicks, R., Weaver M., Horn, B., Xie, W. and Shier, W. (2004). Comparison of cultural and analytical methods for determination of aflatoxin production by Mississipi Delta *Aspergilus* isolates. *Can. Journal. Microbiolology.* 50193199.

Abubakari, S. (2013). Development of Intensity - Duration-Freqency-Curves for Kumasi. MSc. Dissertation, Department of Civil Engineering, College of Engineering, Kwame Nkrumah University of Science and Technology.

Achiaa, N. (2015). Special Report; Role of traditional nika-nika in our food processing chain. ghanaweb.com

Afriyie-Gyawu, E., Wang, P., Ankrah, N.A., Huebner, H.J., Johnson, N.M., Ofosuhene M., Tang, Y., Tang, L., Xu, L., Jolly, P.E., Ellis, W., Ofori-Adjei, D., Williams, J., Wang, J. and Phillips, T. (2008). Novasil Clay Intervention in Ghanaians at high risk aflatoxicosis. *Food additive and Contaminant.*

Ahsan, S., Bhatti I., Asi, M., Bhatti, N. and Sheikh, A., (2010). Occurrence of aflatoxins in maize grains from central areas of Punjab, Pakistan. *International Journal Agriculture Biology*, 12: 571–575

Akrobortu, D. (2008). Aflatoxin Contamination of maize from different storage locations in Ghana, Masters Dissertation, Kwame Nkrumah University of Science and Technology

Anonymous (1967). Limiting temperature and humidity for production of aflatoxin by *A. flavus*in peanuts. *Nutri. Rev*. 25: 286 – 287

AOAC, (2000a). Mycotoxins; aflatoxin17th edition chapter 49, subchapter 2

AOAC, (2000b). Sampling of aflatoxin, preparation of sample, 17th edition 2000, official method 977.16, 972.26a,

AOAC, (2000c). Official method 977.22 standards for aflatoxin, 17th edition

AOAC, (2000d). Offical method 971.22, standard of aflatoxin subpara E, 17th edition

AOAC, (2006). Official Method 2005.08 Aflatoxins in Corn, Raw Peanuts, and Peanut Butter Liquid Chromatography with Post-Column Photochemical Derivatization First Action 2005

Armah, M. (2010). Investment Opportunity in Ghana; Maize, Soya and Rice Production and Process. Millennium Challenge Account report.

Atalla, M., Hassanein, N., El-beih, A. and Youssef, A. (2004). Effect of Fluorescent and UV Light on Mycotoxin Production Under Different Relative Humidities in Wheat Grains. Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Cairo, Egypt, Microbiology Department, Faculty of Science, Ain-shams University, Abbasia, Cairo, Egypt. *International Journal of Agriculture & Biology*. 1560–8530/2004/06–6–1006–1012

Bayman, P. and Baker J. (2006). Ochratoxins: a global perspective. *Mycopathologia*. 215–23.

Bennett, J. and Klich M. (2003). Mycotoxins. *Clinical Microbiology.* 497–516.

Bhadriraju, S. (2013). Sanitation issues in flour mills in Ho Chi Minh, Vietnam. Department of Grain Science and Industry, PhD Kansas State University Manhattan, KS 66506, USA. 4th Annual SE Asia District Conference. http://www.ksre.ksu.edu/grsc_subi

Biollaz, M., Buchi, G. and Milne, G. (1970). Biosynthesis of aflatoxins. *Journal of the American Chemical Society*. 92, 1035-1043.

Boateng, E., Ewusi, K., Kanbur, R. and Mcky, A. (1990). A Poverty Profile for Ghana, 1987-1988. Social Dimensions of Adjustment in Sub-saharan Africa Working Paper No. 5 Washington D. C., the World Bank

Boonen, J., Malysheva, Svetlana V., Taevernier, Lien; Diana Di Mavungu, J., De Saeger, S., De Spiegeleer, B. (2012). Human skin penetration of selected model mycotoxins.

Toxicology. 21–32.

Boutrif, E. (1998). Prevention of aflatoxin in pistachios. *Food, nutrition and agriculture*.

Brase, S., Glaser, F., Kramer, C., Linder, S., Linsenmeier, A., Masters, K., Meister, A., Ruff, B. and Zhong, S. (2013). The Chemistry of Mycotoxins, Progress in the Chemistry of Organic Natural Products, Vol. 97, Doi 10.1007/978-3-7091-1312-7_2

Chang, S.B., Abdel-Kader, M.M., Wick, E.L and Wogan, G.N. (1963). Aflatoxin B_2: Chemical Identity and Biological Activity. *Science Journal.* 29; 142 (3596):1191-2.

Chelule, P., Gqaleni, N., Dutton, M and Chuturgoon, A. (2001). Exposure of Rural and Urban Populations in KwaZulu Natal, South Africa, to Fumonisin B1 in Maize. Environmental Health Research Unit, School of Medical Sciences, Nelson R. Mandela School of Medicine, University of Natal, Congella, South Africa

Chibuike, O.A. (2015). Public Health Significance of Aflatoxin in food industry. 1st Scientific conference of Central and West African Region of Society of Environmental Toxicology and Chemistry (SETAC Africa), Volume 1

Christensen, C.M. and Kaufman, H.H. (1965). Deterioration of stored grains by fungi. *Bot. Rev*. 23: 108-134

Codex Stan (1969). General Principles of Food Hygiene. *CAC/RCP 1-1969* Adopted 1969. Amendment 1999. Revisions 1997 and 2003

EFSA, Journal (2012). Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed

Ellis, W., Smith, J., Simpson, B., Khanizadeh, S. and Oldham, J. (1993). Control of growth and aflatoxin production by *Aspergillus flavus* under modified atmosphere packaging (MAP) conditions. *Food Microbiology, 10:9-21.*

Erbas, M., Arslan, S. and Durak A. (2013). Safety Risks in Flour and Hygiene and Sanitation in Flour Mills for Producing Safe Flour Akdeniz University Faculty of Engineering Department of Food Engineering, Antalya

Essel, K., Asum-Kwarteng, G. and Tseko, V. (2015). Contribution of Commercial Mills to Food Safety in Ghana. Myjoyonline.com

Fact sheet: http://www.micotoxinas.com.br/Aflafacts.pdf

Farhat, J., Ijaz B., Muhammad K., and Muhammad S. (2012). Effect of UVC Irradiation on Aflatoxins in Ground Nut (Arachis hypogea) and Tree Nuts (Juglansregia', Prunusduclus and Pistachio vera). *J. Chem. Soc. Pak.* Vol. 34, No. 6

FDA (2014). Data on small scale manufacturers, Food Premises Inspection Unit (F.P.I.U.) **Fox**, E.M., Howlett, B.J. (2008). Secondary metabolism: regulation and role in fungal biology. *Current Opin. Microbiol.* 481–7.

Ghana Standard (2003). Specification for degermed Maize (corn) meal and maize (corn) grits

Ghana Statistical Service (2010). Population and Housing Census Final Result

Gong, Y, Turner, P., Hall, A, and CP, W. (2008). Aflatoxin exposure and impaired child growth in West Africa In Mycotoxins*:* Detection Methods, Management, Public Health and Agricultural Trade. *CAB International*, 53-65.

Halima, Z., Riyam H., and Amna, M. (2015). Study the effect of ozone gas and ultraviolet radiation and microwave on the degradation of aflatoxin B1 produce by *Aspergillus flavus* on stored Maize grains. *Journal of Agriculture and Veterinary Science.* 2319-2380.

Heather, C. and Snedeker, S. (2004). Ochratoxin-A: Its Cancer Risk and Potential for Exposure. Cornell University Program on Breast Cancer and Environmental Risk factors Facts sheet

Hell, K. (1997). Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin. An unpublished PhD Dissertation, University of Hannover.

Hell, K., Cardwel, K., Setamou M. and Poehling H. (2000). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. J. Stored Prod. Res. 36:365-382

Horn, W.B, Jorge Ramirez-Prado, H., Ignazio Carbone (2009). The sexual state of *Aspergillus parasiticus*. *Mycologia* (*Mycological Society of America*). *101* (2): 275–280. doi*:10.3852/08-205. PMID 19397202.*

Howell, J. (1998). Property: Call time on heavy metal poisoning. *Business, News". The Independent*. Retrieved 2011-08-06.

Hussein, H.S. and Brasel J.M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*.101–34.

Kaaya, N. and Warren H. (2005). A Review of Past and Present Research on Aflatoxin in Uganda: *African Journal of Food Agriculture Nutrition and Development (AJFAND).* vol.5. No.1; pp1-18.

Kumasi Metropolitan Assembly (2013). Composite budget for 2013 fiscal year, Republic of Ghana

Kumi, J., Mitchell, N., Asare, G., Dotse E., Kwaa, F., Phillips, T. and Ankrah N. (2014). Aflatoxins and Fumonisins contamination of home-made food (weanimix) from cereal-legume blends for children in Ejura Sekyedumase District, Ashanti Region, Ghana. *Ghana Medical Journal*. DOI: http://dx.doi.org/10.4314/gmj.v48i3.2

Kwofie, S., Andrews, A. and Mensah, E. (2011). The Quality of Locally-Manufactured Cornmill Grinding Plates. *Journal of Science and Technology*.

Lizárraga-Paulín, E., Moreno-Martínez, E. and Miranda-Castro, S. (2011). Aflatoxins and Their Impact on Human and Animal Health: An Emerging Problem. *Biochemistry and Molecular Biology*. ISBN: 978-953-307-395-8,

Martins, M., Martins H. and Bernardo F. (2001). Aflatoxins in spices marketed in Portugal. *Food Additive Contamination journal.* 315–9.

Mateo, R., Medina, A., Mateo, E., Mateo, F. and Jimenez, M. (2007). An overview of ochratoxin A in beer and wine. *International Journal Food Microbiology*. 119 (1-2), 79-83

Milani, J. (2013). Ecological conditions affecting mycotoxin production in cereals: a review Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

Moss, M. (2008). Fungi, quality and safety issues in fresh fruits and vegetables. *J. Appl. Microbiology.*1239–43.

Nendomachuli, N.E. (2011). Evaluation of decentralization at the sub-district structure level: A case of the Kumasi Metropolitan Assembly. *KNUST journal*

Nielsen, N. L. (2003). Maize: New Uses for an Old Crop. Far Newzealand Maize Conference Proceedings, 12-13 Feb. 2003, Hamilton, New Zealand.

Obioha, I. (1979). Distribution, production, analysis and effects of aflatoxin in animal tissues and effects of scirpenetoxins on chicken embryos. *Retrospective Theses and Dissertations*. Paper 6661

Phillips, D., Afriyie-Gyawu, E., Williams, J., Huebner, H., Ankrah, A., Ofori-Adjei D., Jolly P., Johnson N., Taylor, J., Marroquin-Cardona, A., Xu, L., Tang, L. and Wang, S. (2008). Reducing human exposure to aflatoxin through the use of clay: a review. *Food Additive Contaminant*.134-45. doi: 10.1080/02652030701567467.

Purchase, I.F.H (1967): Food Cosmetic. *Toxicology*. 5, 339.

Richard, J.L. (2007). Some major mycotoxins and their mycotoxicoses—an overview. *International Journal Food Microbiology.* 3–10.

Shivender, S. and Anupreet, K. (2012). Aflatoxin B1: Toxicity, characteristics and analysis: Mini review Global. *Advanced Research Journal of Chemistry and Material Science*. Vol. 1(4) pp. 063-070, 2012

Strosnider, H., Azziz-Baumgartner E., Banziger M., Bhat R.V., Breiman R., Brune M., DeCock K., Dilley A., Groopman J., Hell K., Henry S., Jeffers D., Jolly C., Jolly P., Kibata G., Lewis L., Liu X., Luber G., McCoy L., Mensah P., Miraglia M., Misore A., Njapau H., Ong C., Onsongo M., Page S., Park D., Patel M., Phillips T., Pineiro M., Pronczuk J., Schurz Rogers H, Rubin C, Sabino M, Schaafsma A, Shephard G, Stroka J, Wild C, Williams J. and Wilson, D. (2006). Workgroup Report: Public Health Strategies for Reducing Aflatoxin Exposure in Developing Countries. *Environmental Health Perspectives*. Page 114.

Thomas, R. (1965). Biosynthetic pathways involving ring cleavage. In Biogenesis of Antibiotic Substances.*New YorkAcademic Press*.155-166.

Trucksess, M. and Scott, P. (2008). Mycotoxins in botanicals and dried fruits: A review". *Food Additive Contaminant.*181–92.

Vesonder, R., Beckwith, A., Ciegler, A. and Dimler, R. (1975). Ammonium hydroxide treatment of aflatoxin B1. Chemical characteristics and biological effects. *Journal Agriculture Food Chemistry*. pp 242–243

Wannop, C. (1961). The Histopathology of Turkey "X" Disease in Great Britain. *Avian Diseases*.371–381.

Williams, J., Phillips T., Jolly P., Stiles J., Jolly C. and Aggarwal D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential

health consequences, and interventions. *American Journal of Clinical Nutrition*, 1106–1122

Wu, F. (2010). The global burden of disease caused by foodborne aflatoxin. *WHO Commissioned Report, Foodborne Disease Burden Epidemiology Reference Group (FERG).*

Wu, F. and Khlangwiset P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. *Food Additives & Contaminants.*

Yin, Y., Yan, L., Jiang, J. and Ma, Z. (2008): Biological control of aflatoxin contamination of crops. *Journal Zhejiang University of Science.*787–92.

APPENDICES

APPENDIX 1

GMP CHECKLIST FOR DISC ATTRITION MILLS

AUDITORS NAME : Agbeve Nelson

ID. NO. : L_1

SUBMETRO : Subin

LOCATION : Amekom, Anlo Dzom

DAY OF MAXIMUM SALE : Wednesday

DAY OF MINIMUM SALE : Thursday

TASK

Observe the implementation and management role on food safety using the attached checklist. Score the implementation and then present your findings with recommendation to improve the food safety in/of the factory.

Awarding a score is based on observation, documentary evidence and response given by the operator

GMP Checklist Assessment guide

Unsatisfactory (0 %): No, there is no reasonable evidence that adequately address the check item. This poses serious threat to public health and safety. And needs immediate response. This score is not acceptable.

Improvement required (25 %): Yes, there is evidence that indicate little amount of efforts made to address the check item but not enough to eliminate or minimize the threat to public health and safety

Satisfactory (50 %): Yes, there exist enough evidence that address the check item. But there is still room for improvement. Threat to public health and safety is limited.

Excellent (100 %): Yes, there are reasonable amount of evidence that address the check item. And does not pose any threat to public health and safety. This is the highest score that could be attained

SECTION 1: GENERAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	1.1.1	Does the operator have appropriate training in proper food handling techniques and food protection principles?		√			
	1.1.2	Does the operator report health conditions that might contaminate the food?		√			
	1.1.3	Does the operator protect himself against contamination of food by properly wearing suitable outer garment, hair nets, beard covering etc.?	√				
	1.1.4	Does the operator wash hands thoroughly before work and after each absence from their work station?	√				
	1.1.5	Does the operator remove unsecured jewelry and other objects that might fall into food?		√			

Personnel Management: operator orientation, quality assurance and job training	1.1.6	Does the operator confine eating, drinking, gum chewing and use of tobacco to areas where food is not exposed?	√				
	1.1.7	Does the operator have medical certification and how current is the certificate?		√			
	1.1.8	Does the operator have any formal training in food safety?		√			
TOTAL %			15.625 %				

SECTION 2: FACILITY CONTROL

Section	**N0.**	**Check item**	**Score %**				**Reason/ comment**
			0 %	**25 %**	**50 %**	**100 %**	
			Unsatisfactory	**Improvement required**	**Effective**	**Excellent**	

Facility design and layout: Structure and fabrication	2.1.1	Are the facility buildings and structures suitable to maintain sanitary operations and to produce safe food?	√				
	2.1.2	Does the facility building provide sufficient space for placement of milling equipment to permit maintenance of sanitary operations and prevent cross contamination?	√				
	2.1.3	Does the design of the facility permit the separation of operations in which contamination is likely to occur? How is separation of operations achieved? By location, time, partition, airflow, enclosed systems?	√				
	2.1.4	Does the facility have floors, walls, ceilings constructed in a manner to facilitate adequate cleaning and repair?	√				
	2.1.5	Does the facility have adequate screening or other protection against pest?	√				
	2.1.6	Does the facility have reasonably accessible toilet facility?		√			
2.2 Suitability of Location	2.2.1	Is the facility located in an environment that is free of dust, flood water, fumes from vehicular activities?		√			
	2.2.2	Is the facility constructed in a manner to prevent interference from adjoining facilities?	√				
	2.2.3	Does the facility have entry barriers or restrictions to person or domestic animals?	√				
	2.3.1	Does the facility have adequate lighting?		√			

Environmental and control program	2.3.2	Does the lighting system have safety features so as to prevent them from falling into food products in case of explosion?	√				
	2.3.3	Does the facility have adequate ventilation?	√				
	2.3.4	Does the facility have adequate portable water for its operation?		√			
Facility maintenance and good house keeping	2.4.1	Are the hopper and the grinding discs regularly cleaned of food residues before attending to new clients and how often?		√			
	2.4.2	Does the facility have its surroundings kept free from litter and waste with grass and weeds trimmed?		√			
	2.4.3	Do the drainage systems outside and inside the facility constructed in a manner to prevent contamination of food stuffs?	√				
	2.4.4	Does the facility have a waste disposal system in place adequate enough to protect against contamination?		√			
	2.4.5	Does the facility have a good maintenance culture in place? Are building facilities, fixtures, etc maintained in good state of repairs?		√			
TOTAL %			11.1 %				

SECTION 3: EQUIPMENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Equipment and placement: maintenance program	3.1.1	Are equipment designed to be adequately cleanable and properly maintained?		√			
	3.1.2	Has the equipment been installed in a way that facilitate their cleaning and adjacent spaces?		√			
	3.1.3	Are food contact surface materials made of non-corrosion, resistant and nontoxic material such as stainless steel?		√			
TOTAL %			25 %				

SECTION 4: MATERIALS /COMPONENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	4.1.1	Does the operation of the facility help to identify critical control point and corresponding corrective actions such as metal detectors to remove iron filings from the food?	√				

Materials /component	4.1.2	Are products stored off the floor and away from walls and ceilings to prevent harboring of pest?	√				
TOTAL%			0.00 %				

SECTION 5: OPERATIONAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Operational control	5.1.1	Are the products to be milled ascertained that they are wholesome before milling?	√				
	5.1.2	Is there a formalized program for control of bacteria, yeast and mould?	√				
	5.1.3	Are all the measures and practices at the operation of the facility acceptable for the safety of the food?		√			
TOTAL %			8.33 %				

Conclusions and recommendation for the improvement

……

……
……
……
……
……
……
……
……
……
……
……
……
(Factory visit guidelines ICD-UGL FSNTC, NFS, UG, 2013- modified)

GMP CHECKLIST FOR DISC ATTRITION MILLS

AUDITORS NAME : Agbeve Nelson

ID. NO. : L_2

SUBMETRO : Subin

LOCATION : Adjacent Apostolic Church

DAY OF MAXIMUM SALE : Wednesday

DAY OF MINIMUM SALE : Thursday

TASK

Observe the implementation and management role on food safety using the attached checklist. Score the implementation and then present your findings with recommendation to improve the food safety in/of the factory.

Awarding a score is based on observation, documentary evidence and response given by the operator

GMP Checklist Assessment guide

Unsatisfactory (0 %): No, there is no reasonable evidence that adequately address the check item. This poses serious threat to public health and safety. And needs immediate response. This score is not acceptable.

Improvement required (25 %): Yes, there is evidence that indicate little amount of efforts made to address the check item but not enough to eliminate or minimize the threat to public health and safety

Satisfactory (50 %): Yes, there exist enough evidence that address the check item. But there is still room for improvement. Threat to public health and safety is limited.

Excellent (100 %): Yes, there are reasonable amount of evidence that address the check item. And does not pose any threat to public health and safety. This is the highest score that could be attained

SECTION 1: GENERAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	1.1.1	Does the operator have appropriate training in proper food handling techniques and food protection principles?		√			
	1.1.2	Does the operator report health conditions that might contaminate the food?		√			
	1.1.3	Does the operator protect himself against contamination of food by properly wearing suitable outer garment, hair nets, beard covering etc.?		√			

Personnel Management: operator orientation, quality assurance and job training	1.1.4	Does the operator wash hands thoroughly before work and after each absence from their work station?		√			
	1.1.5	Does the operator remove unsecured jewelry and other objects that might fall into food?		√			
	1.1.6	Does the operator confine eating, drinking, gum chewing and use of tobacco to areas where food is not exposed?		√			
	1.1.7	Does the operator have medical certification and how current is the certificate?		√			
	1.1.8	Does the operator have any formal training in food safety?		√			
TOTAL %			25 %				

SECTION 2: FACILITY CONTROL

Section	**N0.**	**Check item**	**Score %**				**Reason/ comment**
			0 %	**25 %**	**50 %**	**100 %**	
			Unsatisfactory	**Improvement required**	**Effective**	**Excellent**	

Facility design and layout: Structure and fabrication	2.1.1	Are the facility buildings and structures suitable to maintain sanitary operations and to produce safe food?		√			
	2.1.2	Does the facility building provide sufficient space for placement of milling equipment to permit maintenance of sanitary operations and prevent cross contamination?		√			
	2.1.3	Does the design of the facility permit the separation of operations in which contamination is likely to occur? How is separation of operations achieved? By location, time, partition, airflow, enclosed systems?	√				
	2.1.4	Does the facility have floors, walls, ceilings constructed in a manner to facilitate adequate cleaning and repair?		√			
	2.1.5	Does the facility have adequate screening or other protection against pest?		√			
	2.1.6	Does the facility have reasonably accessible toilet facility?		√			
2.2 Suitability of Location	2.2.1	Is the facility located in an environment that is free of dust, flood water, fumes from vehicular activities?		√			
	2.2.2	Is the facility constructed in a manner to prevent interference from adjoining facilities?		√			
	2.2.3	Does the facility have entry barriers or restrictions to person or domestic animals?		√			
	2.3.1	Does the facility have adequate lighting?		√			

Environmental and control program	2.3.2	Does the lighting system have safety features so as to prevent them from falling into food products in case of explosion?	√				
	2.3.3	Does the facility have adequate ventilation?		√			
	2.3.4	Does the facility have adequate portable water for its operation?		√			
Facility maintenance and good house keeping	2.4.1	Are the hopper and the grinding discs regularly cleaned of food residues before attending to new clients and how often?		√			
	2.4.2	Does the facility have its surroundings kept free from litter and waste with grass and weeds trimmed?		√			
	2.4.3	Do the drainage systems outside and inside the facility constructed in a manner to prevent contamination of food stuffs?		√			
	2.4.4	Does the facility have a waste disposal system in place adequate enough to protect against contamination?		√			
	2.4.5	Does the facility have a good maintenance culture in place? Are building facilities, fixtures, etc maintained in good state of repairs?		√			
TOTAL %			13.889 %				

SECTION 3: EQUIPMENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Equipment and placement: maintenance program	3.1.1	Are equipment designed to be adequately cleanable and properly maintained?		√			
	3.1.2	Has the equipment been installed in a way that facilitate their cleaning and adjacent spaces?		√			
	3.1.3	Are food contact surface materials made of non-corrosion, resistant and nontoxic material such as stainless steel?		√			
TOTAL %			25 %				

SECTION 4: MATERIALS /COMPONENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	4.1.1	Does the operation of the facility help to identify critical control point and corresponding corrective actions such as metal detectors to remove iron filings from the food?	√				

Materials /component	4.1.2	Are products stored off the floor and away from walls and ceilings to prevent harboring of pest?		√			
TOTAL %			12.5 %				

SECTION 5: OPERATIONAL CONTROL

Section	**N0.**	**Check item**	**Score %**				**Reason/ comment**
			0 %	**25 %**	**50 %**	**100 %**	
			Unsatisfactory	**Improvement required**	**Effective**	**Excellent**	
Operational control	5.1.1	Are the products to be milled ascertained that they are wholesome before milling?		√			
	5.1.2	Is there a formalized program for control of bacteria, yeast and mould?		√			
	5.1.3	Are all the measures and practices at the operation of the facility acceptable for the safety of the food?		√			
TOTAL %			25 %				

Conclusions and recommendation for the improvement

……

……

……

……
……
……
……
……
……
……
……
……
……
……
……
(Factory visit guidelines ICD-UGL FSNTC, NFS, UG, 2013- modified)

GMP CHECKLIST FOR DISC ATTRITION MILLS

AUDITORS NAME : Richard

ID. NO. : H_1

SUBMETRO : Asokwa

LOCATION : Kaase

DAY OF MAXIMUM SALE : Wednesday

DAY OF MINIMUM SALE : Thursday

TASK

Observe the implementation and management role on food safety using the attached checklist. Score the implementation and then present your findings with recommendation to improve the food safety in/of the factory.

Awarding a score is based on observation, documentary evidence and response given by the operator

GMP Checklist Assessment guide

Unsatisfactory (0 %): No, there is no reasonable evidence that adequately address the check item. This poses serious threat to public health and safety. And needs immediate response. This score is not acceptable.

Improvement required (25 %): Yes, there is evidence that indicate little amount of efforts made to address the check item but not enough to eliminate or minimize the threat to public health and safety

Satisfactory (50 %): Yes, there exist enough evidence that address the check item. But there is still room for improvement. Threat to public health and safety is limited.

Excellent (100 %): Yes, there are reasonable amount of evidence that address the check item. And does not pose any threat to public health and safety. This is the highest score that could be attained

SECTION 1: GENERAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	1.1.1	Does the operator have appropriate training in proper food handling techniques and food protection principles?		√			
	1.1.2	Does the operator report health conditions that might contaminate the food?		√			
	1.1.3	Does the operator protect himself against contamination of food by properly wearing suitable outer garment, hair nets, beard covering etc.?		√			

Personnel Management: operator orientation, quality assurance and job training	1.1.4	Does the operator wash hands thoroughly before work and after each absence from their work station?		√			
	1.1.5	Does the operator remove unsecured jewelry and other objects that might fall into food?		√			
	1.1.6	Does the operator confine eating, drinking, gum chewing and use of tobacco to areas where food is not exposed?			√		
	1.1.7	Does the operator have medical certification and how current is the certificate?		√			
	1.1.8	Does the operator have any formal training in food safety?			√		
TOTAL %			31.25 %				

SECTION 2: FACILITY CONTROL

Section	**N0.**	**Check item**	**Score %**				**Reason/ comment**
			0 %	**25 %**	**50 %**	**100 %**	
			Unsatisfactory	**Improvement required**	**Effective**	**Excellent**	

Facility design and layout: Structure and fabrication	2.1.1	Are the facility buildings and structures suitable to maintain sanitary operations and to produce safe food?		√			
	2.1.2	Does the facility building provide sufficient space for placement of milling equipment to permit maintenance of sanitary operations and prevent cross contamination?		√			
	2.1.3	Does the design of the facility permit the separation of operations in which contamination is likely to occur? How is separation of operations achieved? By location, time, partition, airflow, enclosed systems?		√			
	2.1.4	Does the facility have their floors, walls, ceilings constructed in a manner to facilitate adequate cleaning and repair?		√			
	2.1.5	Does the facility have adequate screening or other protection against pest?	√				
	2.1.6	Does the facility have reasonably accessible toilet facility?		√			
Suitability of Location	2.2.1	Is the facility located in an environment that is free of dust, flood water, fumes from vehicular activities?		√			
	2.2.2	Is the facility constructed in a manner to prevent interference from adjoining facilities?		√			
	2.2.3	Does the facility have entry barriers or restrictions to person or domestic animals?		√			
	2.3.1	Does the facility have adequate lighting?		√			

Environmental and control program	2.3.2	Does the lighting system have safety features so as to prevent them from falling into food products in case of explosion?	√				
	2.3.3	Does the facility have adequate ventilation?		√			
	2.3.4	Does the facility have adequate portable water for its operation?		√			
Facility maintenance and good house keeping 2.4	2.4.1	Are the hopper and the grinding discs regularly cleaned of food residues before attending to new clients and how often?			√		
	2.4.2	Does the facility have its surroundings kept free from litter and waste with grass and weeds trimmed?		√			
	2.4.3	Do the drainage systems outside and inside the facility constructed in a manner to prevent contamination of food stuffs?		√			
	2.4.4	Does the facility have a waste disposal system in place adequate enough to protect against contamination?		√			
	2.4.5	Does the facility have a good maintenance culture in place? Are building facilities, fixtures, etc maintained in good state of repairs?		√			
TOTAL %			23.611 %				

SECTION 3: EQUIPMENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Equipment and placement: maintenance program	3.1.1	Are equipment designed to be adequately cleanable and properly maintained?		√			
	3.1.2	Has the equipment been installed in a way that facilitate their cleaning and adjacent spaces?		√			
	3.1.3	Are food contact surface materials made of non-corrosion, resistant and nontoxic material such as stainless steel?		√			
TOTAL %			25 %				

SECTION 4: MATERIALS /COMPONENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	4.1.1	Does the operation of the facility help to identify critical control point and corresponding corrective actions such as metal detectors to remove iron filings from the food?	√				

Materials /component	4.1.2	Are products stored off the floor and away from walls and ceilings to prevent harboring of pest?		√			
TOTAL %			12.5 %				

SECTION 5: OPERATIONAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Operational control	5.1.1	Are the products to be milled ascertained that they are wholesome before milling?		√			
	5.1.2	Is there a formalized program for control of bacteria, yeast and mould?		√			
	5.1.3	Are all the measures and practices at the operation of the facility acceptable for the safety of the food?		√			
TOTAL %			25 %				

Conclusions and recommendation for the improvement

……

……

……
……
……
……
……
……
……
……
……
……
……
……
……
……
(Factory visit guidelines ICD-UGL FSNTC, NFS, UG, 2013- modified)

GMP CHECKLIST FOR DISC ATTRITION MILLS

AUDITORS NAME : Richard

ID. NO. : H_2

SUBMETRO : Asokwa

LOCATION : Mayanka

DAY OF MAXIMUM SALE : Wednesday

DAY OF MINIMUM SALE : Thursday

TASK

Observe the implementation and management role on food safety using the attached checklist. Score the implementation and then present your findings with recommendation to improve the food safety in/of the factory.

Awarding a score is based on observation, documentary evidence and response given by the operator

GMP Checklist Assessment guide

Unsatisfactory (0 %): No, there is no reasonable evidence that adequately address the check item. This poses serious threat to public health and safety. And needs immediate response. This score is not acceptable.

Improvement required (25 %): Yes, there is evidence that indicate little amount of efforts made to address the check item but not enough to eliminate or minimize the threat to public health and safety

Satisfactory (50 %): Yes, there exist enough evidence that address the check item. But there is still room for improvement. Threat to public health and safety is limited.

Excellent (100 %): Yes, there are reasonable amount of evidence that address the check item. And does not pose any threat to public health and safety. This is the highest score that could be attained

SECTION 1: GENERAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	1.1.1	Does the operator have appropriate training in proper food handling techniques and food protection principles?		√			
	1.1.2	Does the operator report health conditions that might contaminate the food?	√				
	1.1.3	Does the operator protect himself against contamination of food by properly wearing suitable outer garment, hair nets, beard covering etc.?		√			

Personnel Management: operator orientation, quality assurance and job training	1.1.4	Does the operator wash hands thoroughly before work and after each absence from their work station?		√			
	1.1.5	Does the operator remove unsecured jewelry and other objects that might fall into food?		√			
	1.1.6	Does the operator confine eating, drinking, gum chewing and use of tobacco to areas where food is not exposed?	√				
	1.1.7	Does the operator have medical certification and how current is the certificate?	√				
	1.1.8	Does the operator have any formal training in food safety?	√				
TOTAL %			12.5 %				

SECTION 2: FACILITY CONTROL

Section	**N0.**	**Check item**	**Score %**				**Reason/ comment**
			0 %	**25 %**	**50 %**	**100 %**	
			Unsatisfactory	**Improvement required**	**Effective**	**Excellent**	

Facility design and layout: Structure and fabrication	2.1.1	Are the facility buildings and structures suitable to maintain sanitary operations and to produce safe food?	√				
	2.1.2	Does the facility building provide sufficient space for placement of milling equipment to permit maintenance of sanitary operations and prevent cross contamination?		√			
	2.1.3	Does the design of the facility permit the separation of operations in which contamination is likely to occur? How is separation of operations achieved? By location, time, partition, airflow, enclosed systems?	√				
	2.1.4	Does the facility have their floors, walls, ceilings constructed in a manner to facilitate adequate cleaning and repair?	√				
	2.1.5	Does the facility have adequate screening or other protection against pest?	√				
	2.1.6	Does the facility have reasonably accessible toilet facility?		√			
Suitability of Location	2.2.1	Is the facility located in an environment that is free of dust, flood water, fumes from vehicular activities?	√				
	2.2.2	Is the facility constructed in a manner to prevent interference from adjoining facilities?	√				
	2.2.3	Does the facility have entry barriers or restrictions to person or domestic animals?	√				
	2.3.1	Does the facility have adequate lighting?		√			

Environmental and control program	2.3.2	Does the lighting system have safety features so as to prevent them from falling into food products in case of explosion?	√				
	2.3.3	Does the facility have adequate ventilation?	√				
	2.3.4	Does the facility have adequate portable water for its operation?	√				
Facility maintenance and good house keeping	2.4.1	Are the hopper and the grinding discs regularly cleaned of food residues before attending to new clients and how often?			√		
	2.4.2	Does the facility have its surroundings kept free from litter and waste with grass and weeds trimmed?	√				
	2.4.3	Do the drainage systems outside and inside the facility constructed in a manner to prevent contamination of food stuffs?	√				
	2.4.4	Does the facility have a waste disposal system in place adequate enough to protect against contamination?	√				
	2.4.5	Does the facility have a good maintenance culture in place? Are building facilities, fixtures, etc maintained in good state of repairs?		√			
TOTAL %			6.94 %				

SECTION 3: EQUIPMENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Equipment and placement: maintenance program	3.1.1	Are equipment designed to be adequately cleanable and properly maintained?		√			
	3.1.2	Has the equipment been installed in a way that facilitate their cleaning and adjacent spaces?		√			
	3.1.3	Are food contact surface materials made of non-corrosion, resistant and nontoxic material such as stainless steel?		√			
TOTAL %			25 %				

SECTION 4: MATERIALS /COMPONENT CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
	4.1.1	Does the operation of the facility help to identify critical control point and corresponding corrective actions such as metal detectors to remove iron filings from the food?	√				

Materials /component	4.1.2	Are products stored off the floor and away from walls and ceilings to prevent harboring of pest?		√			
TOTAL %			12.5 %				

SECTION 5: OPERATIONAL CONTROL

Section	N0.	Check item	Score %				Reason/ comment
			0 %	25 %	50 %	100 %	
			Unsatisfactory	Improvement required	Effective	Excellent	
Operational control	5.1.1	Are the products to be milled ascertained that they are wholesome before milling?		√			
	5.1.2	Is there a formalized program for control of bacteria, yeast and mould?		√			
	5.1.3	Are all the measures and practices at the operation of the facility acceptable for the safety of the food?		√			
TOTAL %			25 %				

Conclusions and recommendation for the improvement

……

……

……
……
……
……
……
……
……
……
……
……
……
……
……
……
(Factory visit guidelines ICD-UGL FSNTC, NFS, UG, 2013- modified)

APPENDIX 2

Table 4: Labelling codes and results from laboratory analysis of samples on aflatoxin

Day of maximum sale (Wednesday)					
	Low income earners $_1$	Low income earners $_2$	High income earners $_1$	High income earners $_2$	Control
Morning Results/ppb	L_1-Max-Morn	L_2-Max-Morn	H_1-Max-Morn	H_2-Max-Morn	C-Max-Morn
	103.26	100.76	90.77	88.32	74.05
Peak period Results/ppb	L_1-Max-Pk	L_2-Max-Pk	H_1-Max-Pk	H_2-Max-Pk	C-Max-Pk
	88.89	120.65	104.27	115.45	74.26
Day of minimum sale (Thursday)					
	L_1	L_2	H_1	H_2	Control
Morning	L_1-Min-Morn	L_2-Min-Morn	U_1-Min-Morn	U_2-Min-Morn	C-Min-Morn
Results/ppb	110.79	119.46	79.39	76.71	65.77

Peak period	L_1-Min-Pk	L_2-Min-Pk	U_1-Min-Pk	U_2-Min-Pk	C-Min-Pk
Results/ppb	158.87	84.15	79.02	80.55	66.19

APPENDIX 3

List of images taken during the realisation of the project

Plate 10: Weighing the samples and sodium chloride into the centrifuge tube

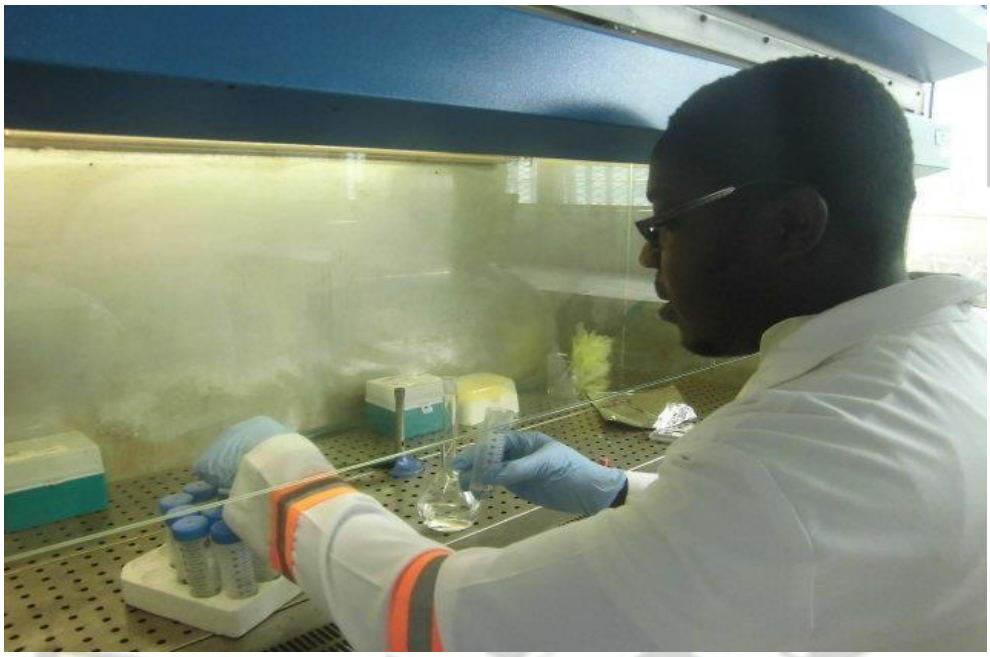

Plate 11: Addition of the acetonitrile to the mixture before vortex

Plate 12: One of the facilities located near a public toilet

APPENDIX 4

Formula for calculating levels of aflatoxin

Aflatoxin, ng/g = **A x (T/I) x (1/W)** Where:

A = ng of aflatoxin as eluate injected **T** = final test solution eluate volume (µL)

I = volume eluate injected into LC (µL) **W** = mass (g) of commodity represented by final extract

Note: **T = 2000** **I = 100** **W = 1**

APPENDIX 5
Statistical analysis of data using minitab software

One-Sample T: Control

```
Test of mu = 20 vs not = 20

Variable  N   Mean     StDev   SE Mean     95% CI          T       P
Control   4   70.07    4.72    2.36        (62.55, 77.58)  21.20   0.000
```

One-Sample T: max-morn

```
Test of mu = 74.05 vs not = 74.05

Variable  N    Mean    StDev   SE Mean       95% CI            T       P max-morn
4     95.78   7.34    3.67          (84.10, 107.45)   5.92    0.010
```

One-Sample T: max-pk

```
Test of mu = 74.26 vs not = 74.26

Variable  N     Mean     StDev   SE Mean   95% CI            T      P max-pk
4     107.32   14.06   7.03     (84.95, 129.68)    4.70   0.018
```

One-Sample T: min-morn

```
Test of mu = 65.77 vs not = 65.77

Variable  N   Mean    StDev   SE Mean   95% CI          T      P min-morn
4    96.6    21.7    10.9      (62.0, 131.2)   2.84   0.066
```

One-Sample T: min-pk

```
Test of mu = 66.19 vs not = 66.19

Variable  N    Mean    StDev   SE Mean   95% CI          T      P min-pk
4    100.6   38.9    19.4      (38.8, 162.5)   1.77   0.174
```

Two-Sample T-Test and CI: L, H

```
Two-sample T for Low income earners vs High income earners

       N   Mean     StDev    SE Mean
L      8   110.9    23.4     8.3
H      8   89.3     13.9     4.9
```

```
Difference = mu (L) - mu (H)
Estimate for difference:  21.54
95% CI for difference:  (0.37, 42.71)
T-Test of difference = 0 (vs not =): T-Value = 2.24  P-Value = 0.047  DF = 11
```