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LEVELS OF OCHRATOXIN A AND AFLATOXINS IN COCOA BEANS FROM FOUR COCOA GROWING REGIONS IN GHANA

A thesis submitted to the Department of Food Science and Technology in partial fulfilment of the requirement for the award of the degree of Master of Science in Food Quality Management

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MSc. except for references and quotations which have been duly acknowledged. Also, to the best of my knowledge, it contains

no materials previously published by another person nor material which has been submitted for the award of degree in a University.

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DEDICATION

I dedicate this work to my parents, Mr & Mrs. Asiedu for the love, encouragement and support given me. I really do cherish you.

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I am most grateful to the Almighty God for the grace and mercies shown me that has led to the successful completion of this work.

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ABSTRACT

Ochratoxin A (OTA) and aflatoxins are important mycotoxins in the food industry. They are produced by toxins producing fungi which contaminate agricultural produce either on farm, after harvesting (during storage) or during transport depending on the environmental condition of exposure. Processing of cocoa into semi-finished and finished products does not completely eliminate OTA and aflatoxins hence measures should be put in place to prevent contamination of these toxicants. In this study sixty samples (thirty shelled and thirty unshelled cocoa beans) were obtained from districts in four cocoa growing regions in Ghana namely Western-South, Western-North, Ashanti and Central regions. Samples were extracted and purified before analysis. A modified QuEChERS-HPLC method with fluorescence detector was used to quantify the mycotoxins of interest. The results showed that no OTA or aflatoxins were detected under the experimental conditions. Results were identical for both shelled and unshelled beans. These results suggest that cocoa beans cultivated from the districts on under study satisfy international limits, and after further

processing for consumption will not cause any likely health implications and economic loss from mycotoxin contamination.

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ABBREVIATIONS

COCOBOD	Ghana Cocoa Board
OTA	Ochratoxin A
CEXP	Cocoa Export
QCBP	Quantity of cocoa bean production
WCF	World cocoa foundation
IFDC	International Development Fertilizer Center
IARC	International Agency for Research on Cancer
JECFA	Joint Expert Committee for Food Additives and Contaminants
PTWI	Provisional Tolerable Weekly Intake
CEC	Commission of the European Communities
FAO	Food and Agricultural Organization
WHO	World Health Organization

CABI	Centre for Agriculture and Bioscience International
EU	European Union
ERP	Economic Recovery Program
CRP	Cocoa Rehabilitation Program
CMC	Cocoa Marketing Company
EC	European Communities
CA	Codex Alimentarius
GAP	Good Agricultural Practices
LOD	Limit of Detection

CHAPTER ONE 1.0 INTRODUCTION

1.1 Cocoa's economic importance

Cocoa is a major agricultural asset in Ghana due to its massive contribution in terms of foreign exchange as well as being a source of employment for citizens. It is the highest foreign exchange earner among all the agricultural produce cultivated in Ghana, hence of great interest to policy makers (Codjoe *et al.*, 2013). Cocoa contributed approximately 3.4% to the total gross domestic product yearly and also accounted for about 29% of the total export revenue generated between the periods of 1990 and 1999 (Anon, 2001). Within the period of 2000 and 2003 also, cocoa contributed an average of 22% of the total export revenue generated (Anon, 2003). The economic relevance of cocoa in Ghana has attracted policy makers again due to its contribution to the total Gross Domestic Product (GDP) (Codjoe *et al.*, 2013). According to Dankwah and Verter, (2014), the CEXP is a positive determining factor for cocoa production (QCBP) with a statistical significance level of 5%. A further elaboration indicates that a 1% increase in the amount of cocoa exported within a year can possibly give rise to a 0.28% increase in cocoa production.

1.2 Mycotoxins in agricultural products

Mycotoxins are secondary metabolites of filamentous fungi. Relatively, mycotoxins are of smaller molecular weight (Bennett, 1987).

The toxicity of fungal contamination depends on the target as well as the concentration of the metabolite involved. Mycotoxins remain harmful to some animal species and even vertebrates in lower concentrations (Graniti, 1972). It is of great concern because of its harmful nature. Scientists have categorized their harmful health effects as being carcinogenic, mutagenic, genotoxic,

teratogenic etc as experienced by humans and some animals after ingestion or inhalation (Richard, 2007).

More than three hundred mycotoxins have been identified and subsequently reported all over the world. Extensive research and analytical studies have identified Aflatoxins, Ochratoxin A, Zearalenone ,Fumonisins amd deoxynivanelol as relatively common due to their natural distribution (IARC, 1993).

Cocoa beans can become susceptible to fungi contamination during certain stages of processing on the farm. Although cocoa beans could be contaminated by ochratoxins during fermentation, predominantly, fungi contamination occurs during the drying and storage stages (Copetti *et al.*, 2010).

Studies by Copetti *et al*., (2013) indicated that ochratoxin contamination is comparatively prevalent in the non-fat portions of the cocoa beans. Results from their research of natural ochratoxin contamination in cocoa products showed that cocoa butter was least contaminated compared to higher levels of contamination in the cocoa shells and cocoa powder.

1.3 Problem statement and Justification

Food safety is very important in the entire food chain. Bacteria happens to be the greatest hazard that compromises food safety followed by mycotoxins (Bankole and Adebanjo, 2003). Mycotoxins which are found in foods contaminated with fungi could be present in cocoa and this is a result of the fact that cocoa is mainly grown in the Central and Western parts of Africa where the climatic condition is characterized by high temperatures and humid areas which are favourable for the growth of fungi specie and subsequently mycotoxins (Oyetunji 2006; Sanchez-Hervas *et al*., 2008).

Mycotoxins have attracted attention in the international front because in addition to its negative impact on economy as a result of agricultural losses it generates, it has adverse health implications on humans as well when consumed (Bhat and Vashanti, 1999).

Within the high range of identified mycotoxins, Ochratoxins and Aflatoxins have become toxins of major interest and focus of studies and this is predominantly due to toxicity and high prevalence. Taking off the shells of cocoa beans by hand has proved to reduce ochratoxins A contamination to an extent (Amezqueta *et al.*, 2005). It is however important to note that there are regulatory measures that have been put in place with respect to Aflatoxins occurrence in products worldwide (Commission of the European Communities, 1998).

The FDA in the US have set a standard specification of 20µg/kg as the maximum residue limit if present in food intended for human consumption with the exception of milk (FAO, 1996).

Aflatoxin regulation from the European Union is more stringent and has set maximum limit of 2µg/kg and 4µg/kg of Aflatoxin B_1 and total aflatoxins respectively in cereals and nuts intended for human consumption (CEC, 1998). This regulation was enforced in January, 2001. The World Bank has estimated that the policy change by the European Union will decrease overall imports of dried fruits, nuts and cereals by 64% in nine African countries namely; Senegal, Gambia, South Africa, Sudan, Mali, Zimbabwe, Egypt and Chad. This loss can be translated into an economic loss of approximately 670 million US dollars in trade per annum (Kellerhals, 2000).

The joint expert committee on Food Additives of FAO and WHO have set provisional maximum limit of intake by humans to be 100ng/kg. On the other hand, the Scientific committee of the European Union have also proposed a maximum limit of daily consumption of 5ng/kg (WHO, 1996).

Studies have shown that the co-existence of Ochratoxin A and Aflatoxin B_1 can increase the mutagenicity of Aflatoxin B_1 (Sedmikova *et al.*, 2001).

Ochratoxin A is a known possible contaminant of cocoa beans and cocoa products exported from some countries in West Africa. With respect to this information, industries involved in cocoa production have been mandated to implement strict preventive measures in order to avoid cocoa contamination by Ochratoxin A (CABI, 2001).

The fact that Ghana happens to be one of the world's largest exporter of cocoa and cocoa also being the only agricultural product to generate the highest foreign exchange to the country Ghana demands that cocoa produced in Ghana should meet all food safety requirements and standards internationally. This include monitoring the ochratoxins as well as aflatoxins levels in cocoa cultivated in the country.

1.4 Objective of the Study The study objective is:

i. Determine the presence and levels of Ochratoxins A and Aflatoxins in dry cocoa beans sampled from four cocoa growing regions in Ghana namely; Ashanti, Western-South, Western-North and Central Regions

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Origin of cocoa in the world

The genus Theobroma of which *Theobroma cacao* is a specie originated from Amazon and Orinoco basins and later spread to the regions of South America, particularly, Mexico. The Olmec and Mayas later termed "the food of gods". Although Theobroma has 22 species the most common is the *Theobroma cacao*. *Amelando cacao* from Brazil was cultivated in the year 1822 in Principe, then in 1830 at Sao Tome, in 1854 at Fernando Po. It was later cultivated in Nigeria and Ghana in the years 1874 and 1879 respectively (Pohlan and Diaz Perez, 2010).

2.2 History of cocoa cultivation in Ghana

Cocoa gained entry to Ghana, then Gold Coast in the year 1876 when it was introduced by Tetteh Quarshie from Fernando Po. The Governor of the then Gold Coast in 1890 established the Aburi botanical gardens with the objective of promoting the large scale cultivation of cocoa for exports. First exports recorded in 1891 was 80lbs which gradually grew to 40,000 tons by 1911 and 240,000 by 1929 (Frankel, 1974). It very important to note that Ghana happened to be the world's largest producer of cocoa within the period of 1911 and 1976 (Darkwah, 2014). Cocoa production in Ghana has gone through four different phases; its introduction and high growth rate (1888-1937), period of stagnation and a subsequent short term of fast, increased growth rate after the country gained its independence (1938-1964), the third phase was characterized by an almost crumpling cocoa economy (1965-1982). The fourth phase was more of recouping and extension and has been the case of the cocoa sector till date (1983 to date). The fourth phase led to the emergence of the Economic Recovery Program (ERP).

Cocoa cultivation first started in the Eastern region, however following the infestation of the crop by the swollen shoot virus and other pests in the Eastern region during the 1940's, cocoa cultivation

shifted to the Western and Brong-Ahafo regions (Amanor 2010). In the mid part of 1940s, cocoa production increased but this time around in the western region. In 1947, Cocoa Marketing Board, currently CMC was established and given the sole responsibility with respect to cocoa purchases.

In 1984, the cocoa sector experienced the emergence of new varieties of cocoa in its hybrid forms by the aid of Cocoa Rehabilitation Program (CRP), a government agency. The hybrid varieties have a greater output as compared to the native "Amelonado" and "Amazons" varieties due to the fact that the hybrid varieties produce fruits within the periods of two to three years where as the older varieties require at least five years to bear fruit. The hybrid varieties are also characterized by the production of more pods per unit tree. There are some relative disadvantages linked to the cultivation of the hybrid varieties. For instance, their cultivation require the practice of new planting procedures, chemicals usage, pruning and more harvesting frequencies at the commencement and end of crop season (Boahene *et al.*, 1999, Bloomfield and Lass, 1992). Within this same period some policy changes were implemented which included; an increase to the prices paid to Ghanaian farmers as compared to farmers in West African countries close by. This was enough motivation to reduce and possibly prevent the rate of smuggling cocoa to neighboring countries.

In 1992, COCOBOD relinquished its responsibility to purchase cocoa locally to six private licensed institutions who are popularly referred to us licensed buying companies (LBCs).

Production recovered again in the period 1995-1996.

The year, 2001 began with a striking growth in the cocoa sector. Good farm practices (example, Application of fertilizers) coordinated by COCOBOD, increased farmers share and increased world prices together positively impacted this significant growth (Vigneri and Santos, 2008). A

fraction of the growth experienced could also be attributed to large volumes of cocoa; approximately 150, 000 tons smuggled from Cote d'Ivoire within the year, 2003-2004 (Brooks *et al*., 2007).

Cocoa cultivated in Ghana is comparatively of higher quality due to low proportions of defective beans, low levels of debris and also a fat content which is slightly above average fat content of cocoa beans from other countries (Agrisystems Ltd. 1997).

Presently there are seven regions well noted for growing cocoa. These include Western South, Western North, Eastern, Ashanti, Brong-Ahafo, Central and Volta regions.

2.3 Brief Description of Cocoa.

The cocoa tree which goes by the botanical name *Theobroma cacao L.* is a tree mainly cultivated for the rich purpose of its beans commonly called cocoa beans. The tree is of height range of 8 to 12 metres (Fowler, 1999).The cocoa tree can be categorized into three main kinds: "Forastero" which is characterized by the yellow colour it usually possesses; "Criollo" which is relatively more expensive and preferred characterized by red or red-orange when it reaches its maturity stage; "Trinitario" is a resultant hybrid of the "Forastero" and "Criollo" varieties. However the dominant variety with respect to the West and Central parts of Africa is the Forastero (Adabe and NgoSamnick, 2014).

2.4 Economic Importance of Cocoa Worldwide and in Ghana

Studies by World Cocoa Foundation (WCF) has shown that averagely five to six million farmers in Latin America, Asia and Tropical Africa are engaged in 90% cultivation of cocoa globally. In

addition, about 40 to 50 million people in the world are dependents of cocoa for living (World Cocoa Foundation, 2014). The Central and Western regions of Africa are able to generate export revenue of about eight billion dollars (IFDC, 2014). Currently, Ghana happens to be the third world's largest producer of cocoa.

Cocoa production has provided employment for Ghanaians. The cocoa industry has been able to employ about 70% of the national agricultural labour (COCOBOD, 2013). The beneficiaries (cocoa farmers) hence receive about 70 to 90 percent of their yearly income from cocoa production (Anang *et al*., 2013; Nunoo *et al*., 2014).

Although Ghana happens to be one of the world's major producer of cocoa, relatively it has lower yields as compared to Malaysia and Ivory Coast which are also major cocoa producers. For instance where as Malaysia and Ivory Coast have average cocoa yields of $1800kgha^{-1}$ and $800kgha^{1}$ respectively, Ghana has an average cocoa yield of $360kgha^{-1}$ (Anon, 1999).

Ghana is also recommended for its production of very high quality cocoa and is able to gain a premium price for its quality cocoa in the international market (Agrisystems Ltd. 1997).

2.5 Health benefits of cocoa

In years past, due to the health benefits of cocoa it was popularly termed the drink of gods and that assertion contributed to its generated scientific name; Theobroma cacao where theo is a greek word translated as god and broma also drink (Latif, 2013).

Cocoa is known to have an antidiabetic property. The mechanism of this property arise from its flavanol content which has the ability to possibly lower insulin resistance by improving NO bioavailability. This function of flavanol is essential due to the fact that insulin sensitivity has a partial dependence on NO bioavailability (Konopatskaya *et al.*, 2003).

A recent research on neurons which emphasized on both young, healthy individuals involving the usage of magnetic resonance imaging technique suggested that ingestion of cocoa contributes to an increase in cerebral blood flow (Francis *et al.*, 2006). This effect of cocoa has also been affirmed by Walters *et al*, 2012. Thus, cocoa can possibly have a possibly have a positive impact in the management and treatment of cocoa (Larrson *et al.*, 2012).

Cocoa has an antistress effect. A research in Switzerland to study the relationship between cocoa consumption and stress levels (Martin *et al.*, 2009). The antistress property of cocoa is due to its ability to stimulate the production of the hormone serotonin which is a soothing neurotransmitter (Benton, 1999). Another hormone; endorphins found in cocoa make humans less sensitive to the feel of pain

Studies have shown that cocoa and its products contain the anti-oxidants; polyphenols, procyanidins, catechins and epicatechins which inhibits reactive oxygen species (Visioli *et al.*, 2000). The presence of polyphenols in cocoa beans function as anti-oxidants which provide cocoa with its anti-carcinogenic (Rodríguez-Ramiro *et al.*, 2011; Oleaga *et al.*, 2012).

Although cocoa is known to have numerous health benefits, it is important to consume it in moderate portions (Kelishadi, 2005).

2.6 Factors affecting cocoa production

There are quite a number of studies that have been undertaken by researchers on the factors that contribute to either a positive or negative impact on cocoa production.

Studies by Fadipe *et al.,* (2012) in Nigeria gave a positive correlation between the size of farm and the subsequent output of coca production. In a similar study conducted in Ghana, Vigneri (2007) showed the cause and effect relationship with respect to farmlands cultivated and their subsequent cocoa production levels.

Abdulai and Rieder (1996) in their study found out the supply of cocoa was directly affected by the supply of finished products, the country's actual exchange rate and the real producer price of cocoa. Boansi in 2013 also released a paper which indicated the quantities of cocoa produced in Ghana had a positive correlation with exchange rate, ratio of real world price to that to that real producer price, real producer price, nominal rate of assistance and foreign direct investment.

In Nigeria, it was found out that their large volumes of output could be firmly attributed to the rates of export in the country (Ndubuto *et al.,* 2010).

Studies carried out by Dorman *et al.,* (2004) in three different cocoa communities in Ghana namely showed that the farmers could link socio-economic factors as playing a role to affect the output of cocoa production. In their opinion, factors such as lack of social amenities like electricity could affect labour due to the migration of the youth to the cities and subsequently investment by cocoa farmers to maintain the cocoa farms. They could also attribute persistence of pests and diseases as significantly affection cocoa production. Although the farmers demonstrated adequate knowledge about the effect of the pests and diseases on the overall output, some couldn't do much to control

the infestation of pests and disease because of high prices associated with hiring or purchasing spraying equipment and pesticides in addition to the extra labour cost involved.

The low rate of tree re-planting also affects cocoa production, farmers usually find it more economical to invest in farm expansion than replacing very old and diseased trees (Ruf, 2001).

Climate plays a major role in the cultivation of cocoa. Due to climate changes experienced in the country, currently Western region provides the bulk of cocoa output. Cocoa is very much sensitive to climate change taking into consideration exposure to sun for long hours, water application/rainfall and temperature due to the impact on evapotranspiration. Climate change also comes with the potential to induce changes in the developmental stages and rates of cocoa pests, their resistance and interaction. This could have adverse impact on crop yield (Codjoe *et al.*, 2013).

Cocoa cultivation is quite delicate due to the several factors that tend to impact on its success. The cocoa tree is susceptible to varying weather conditions as a result of either excessive rains or drought which could possibly have a negative impact on the success of cocoa production and its subsequent yield. For instance the major decline in the early 1980s according government was unfavorable weather conditions causing massive spread of bushfires and subsequently destroying several cocoa farms. Cultivation of cocoa varieties that have relatively low yields and poor farm practices that do not help maintain the farmlands well also negatively affects cocoa outputs (Anon, 1999). Insects and diseases are also factors that lead to low productivity in cocoa cultivation.

Aneani and Ofori-Frimpong (2013) in their research confirmed that eradication of black pod disease by the spraying of fungicides had a major impact on cocoa output in Ghana. Cocoa farmers are likely to face certain challenges such as poor soil fertility, ageing trees and lack of incentives in the form of finance (Darkwah, 2014). Consistently, there is very high demands of cocoa and

cocoa products all over the world and this has helped channeled a lot of funds globally in order to boost cocoa production (World Cocoa Foundation, 2014).

2.7 Description of cocoa and it's cultivation

The cocoa plantation can thrive within the period of 15 to 40 years. Internationally, there are three major regions of cocoa cultivation namely:

- South Asia and Oceania (Malaysia, Papua New Guinea, Indonesia etc.)
- The Gulf of Guinea (Nigeria, Cameroun, Cote D'Ivoire, Ghana etc)
- Central America, South America and Caribbean (Peru, Brazil etc)

The height of the cocoa can extend from 8 to 12 meters (Pohlan and Diaz Perez, 2010). The cocoa seeds commonly known as cocoa beans are found in numbers of about thirty or forty embedded in a pulp in containment called pods. The cocoa pods are of oval shape with a length range of between 12 and 30 centimetres (Schwan and Wheals, 2004; Lima, Almeida, Rob Nout, and Zwietering, 2011). About 14 to 28 pods are needed to generate a kilogram of dried cocoa beans. Nutritionally, the pulp is rich in the sugars; glucose, sucrose and fructose (Lefeber *et al*., 2010). It is highly acidic (Guehi *et al*., 2010) and of a protein composition within the range 0.4 to 0.6% w/w (Lima *et al*., 2011).

The climatic conditions suitable for cocoa cultivation is basically that of the rainforests. Cultivation can be efficiently done in temperature variation within 30-32^{o}C and 18-21^{o}C minimum with the optimum temperature being 25-28^{o}C. High temperatures exceeding 32^{o}C during blossoming lasting for three months in the dry season results in termination of flowers and fruits. The cocoa plant remains vulnerable during the drought and its growth is dependent on evenly

distributed monthly rainfall pattern. The bean size is negatively affected by drought. Hot winds increases the rate of evapotranspiration where as strong winds decreases the leaf area, retarding the development of flowers and fruits (Pohlan and Diaz Perez, 2010).

Environmentally, the cocoa tree requires moist and hot conditions for good cultivation. Under extended drought, the tree's reproductive and vegetative functions becomes depreciated (CacaoNet, 2012).

Cocoa has certain properties which contributes to its quality attribute. The bean count which reflects the consistency in the size the beans, the fat and moisture content defines the status of the cocoa beans in terms of quality. Other phases of the cultivation process which could impact on the quality of beans include transport of wet beans, fermentation, drying and storage.

2.8 The three categories of cocoa tree

The three major varieties of cocoa are Forastero, Criollo and Trinitario. There is also a fourth variety not so common and grown in Ecuador by the name Nacional (Afoakwa *et al.*, 2011a; Amoye, 2006; Awua, 2002; Bartley, 2005; Beckett, 2000; Counet *et al.*, 2004; Motamayor *et al.*, 2008). It is important to note that these varieties are distinct in their percentage yields, appearance of pod as well as the ability to resist diseases and pests (Adeyeye *et al.*, 2010; Afoakwa *et al.*, 2008; Afoakwa, 2010).

Forastero contributes to ninety-five percent of cocoa production worldwide (Saltini *et al.*, 2013) and is commercially referred to as the "bulk cocoa". Forastero find its origin from the Upper Amazon basin. It is a robust tree and beans produced are of a strong flavor. It has pods that are characterized by the yellow, red, orange and purple colours. The cocoa beans of the forastero

category have intense dark purple colour and morphologically have a flat shape compared to that of Criollo. In further comparisons with Criollo, Forastero beans do not have spicy and fruity traits. They are predominately preferred to by chocolate makers to create a rich chocolate flavor blend (Pohlan and Diaz Perez, 2010). They are highly productive with a moderate resistance to dieases and pests (Bartley, 2005; Ferrao, 2002; Lima *et al.*, 2011). The Forastero cultivar is mostly cultivated in West Africa (essentially in Ghana, Cameroun, Ivory Coast and Nigeria) and in Brazil. The seeds of Forastero are flat, having a purple colour and an astringent taste. Relatively, cocoa consumer products manufactured from Forastero beans are less acidic, less bitter and less astringent. This is as a result of its high pH after fermentation and drying (Clapperton *et al.*, 1994; de Muijnck, 2005; Sukha *et al.*, 2008).

Criollo, another category of the cocoa tree is originally grown in the Southern, Central and Northern parts of America. This type is quite unpopular because of its vulnerability to diseases, hence its production is restricted to some parts of Asia and also Central America (Fowler, 1999, Ferrao, 2002; Thompson *et al.*, 2007). They are relatively not very robust, giving rise to softer pods and the pods are characterized by the yellow, white and green colours. Unlike forastero, have less disease resistance potential hence not patronized by most farmers (Pohlan and Diaz Perez, 2010). Physically, Criollo beans are white to ivory in colour. These beans are reported to have a low pH which influences its flavor profile (Ortiz de Bertorelli *et al.*, 2009). Chocolate manufactured from Criollo beans not very rich but characterized by a sophisticated flavor profile hence the Criollo beans are termed “flavor beans” (Pohlan and Diaz Perez, 2010).

The Trinitario category of cocoa beans which is a hybrid of both Forastero and Criollo beans originated from Trinidad (Fowler, 1999; Ferrão, 2002). The tree is mainly cultivated in Central America, Columbia, Papua Guinea as well as Cameroun. As suggested in the name, the Trinitario

category of cocoa beans originated from Trinidad. The beans have a morphological figure of being flat with a purple colour when cut cross-sectionally. The beans from this category vary in colour. Due to its unique flavor just as Criollo, they together have richly enhanced the flavor of chocolate. However, like Forastero, it is also a major cocoa variety cultivated throughout the world presently (Pohlan and Diaz Perez, 2010). The Trinitario type have an intermediate vulnerability to pests and diseases compared to Forastero and Criollo (Fowler, 1999; Ferrão, 2002; Bartley, 2005). One unique characteristic of Trinitario beans uncommon to the other categories is its peculiar winery aroma (Afoakwa *et al*., 2008).

2.9 Harvesting

After growing cocoa, the initial harvest takes place approximately after three years for the hybrid variety and approximately four to five years for the traditional variety. The cocoa tree can produce harvest twice a year and can last for thirty years (Adabe and Ngo-Samnick, 2014). The harvests are the main harvest and the mid which takes place six months following the main harvest. The major harvesting commences at the end of the wet season and is likely to last for three months. Ripe pods can either be harvested with long poles (with cutting edge) when the pods are high or machete. Harvested pods are then categorized in grades based on the quality parameters and placed into piles after which they can be split into halves using wooden calves or machete. A ripe pod is determined by the colour change which is dependent on the cultivar cultivated. One feature of a ripe pod is for the seeds and pulp to be detached from the husk producing a unique sound whenever the cocoa pod is tapped. Ripe cocoa pods are harvested within the duration of seven to fourteen days (Pohlan and Diaz Perez, 2010).

During harvesting, caution should be taken not to harvest unripe pods as beans from unripe pods encounter difficulties in fermenting, in addition pods with fungal infections should be separated and subsequently discarded. Harvesting should be done sequentially and regularly to prevent harvest over ripe pods which are susceptible to fungal infections translating to fungal infested cocoa beans (AusAID, 2010).

After harvesting, cocoa pods are to be stored in a cool and dry environment free from rain or other form of moisture so as to avoid possible fungal growth. Pods can then be broken within a day to a week after harvesting to remove the beans. Pods are to be split open in a manner in which cocoa beans are not damaged as this will affect the overall quality of the cocoa bean after fermentation and drying. Cocoa pods are not to be split open under rains as this will result in the washing away of the pulp that encapsulates the cocoa beans and will subsequently affect fermentation. After breaking of pods, flat and black beans are to be separated and discarded in order not to add it to the entire cocoa beans removed from the pods.

Plate 2.1 Harvesting Of Ripe Cocoa Pod (Source: https://cocoanibs.files.wordpress.com)

Plate 2.2 A Ripe Cocoa Pod Split open (https://bloguldeciocolata.files.wordpress.com/)

2.10 Fermentation of cocoa beans

Fermentation of cocoa beans is a batch process. After beans are removed from the cocoa pods, they are collected with the whitish mucilaginous sweet pulp in heaps, boxes or baskets as per the type of fermentation process that is preferred. In case of fermentation type that is chosen, the cocoa beans are to be placed on banana leaves and also covered with banana leaves or jute bags. This action of covering cocoa beans undergoing fermentation with either banana leaves or jute bag is to prevent passage of excess air into fermenting cocoa and also to prevent loss of moisture in excess

which will result in improper fermentation. Covering the fermenting cocoa also inhibits the loss of heat during fermentation. High temperatures attained during the fermentation is process is necessary for proper and complete fermentation. In this regard, because jute bags are better insulators against heat loss, it is better to combine the jute bags together with the banana leaves and not use the banana leaves in isolation as a covering. Temperature monitoring is required therefore farmers are advised to monitor the temperatures attained during fermentation with a thermometer. Temperatures within the range of 45 to 50C noted to aid in proper fermentation. Relatively, cocoa beans are of better quality when temperature attained during fermentation is closer to 50C. In all types of fermentation too, the beans should be mixed together on preferably the second day and this is to aerate the fermenting beans and increase temperature within also for overall evenly fermented beans (AusAid, 2010).

Fermentation of Criollo type of cocoa beans takes place within the period of three to four days under optimal conditions due to the low levels of polyphenolic compounds. On the other hand, Forastero and Trinitario types require six to eight days to ferment (Pohlan and Diaz Perez, 2010). After fermentation, the beans colour changes from white to brown. Cocoa beans that have undergone proper fermentation are without mold, with a shiny appearance and also their cotyledons easily break (Adabe and Ngo-Samnick, 2014).

The main purpose of fermentation in the entire cocoa processing chain is to develop the chocolate flavor and aroma of cocoa for subsequent chocolate production.

2.10.1 Types of cocoa beans fermentation

Heap fermentation involves the heaping of cocoa beans unto banana leaves layer on the ground. It the commonest type of fermentation conducted in West Africa. It remains the simplest type of fermentation too in that it does not require the use of neither fermentation boxes nor baskets. After heaping the beans on the banana leaves layer, the beans are then covered with a combination of banana leaves and jute bags to help retain the required temperatures.

Plate 2.3 Heap fermentation of cocoa beans (AusAid, 2010)

Basket fermentation is another type of fermentation. In this fermentation process, the basket sides and bottom are lined with banana leaves as well as the top. This inhibits the drying out of the cocoa beans and also insulates the containment against heat loss so as to hold the heat within. The beans can either be placed in the basket just after breaking the pods or can also be spread out on a plastic sheet for a duration of two hours before being transferred into the basket. The latter mode of

treatment results in a rapid increase of temperature and is preferred for better bean quality. Basket fermentation is not appropriate for beans quantity of less than 25kg.

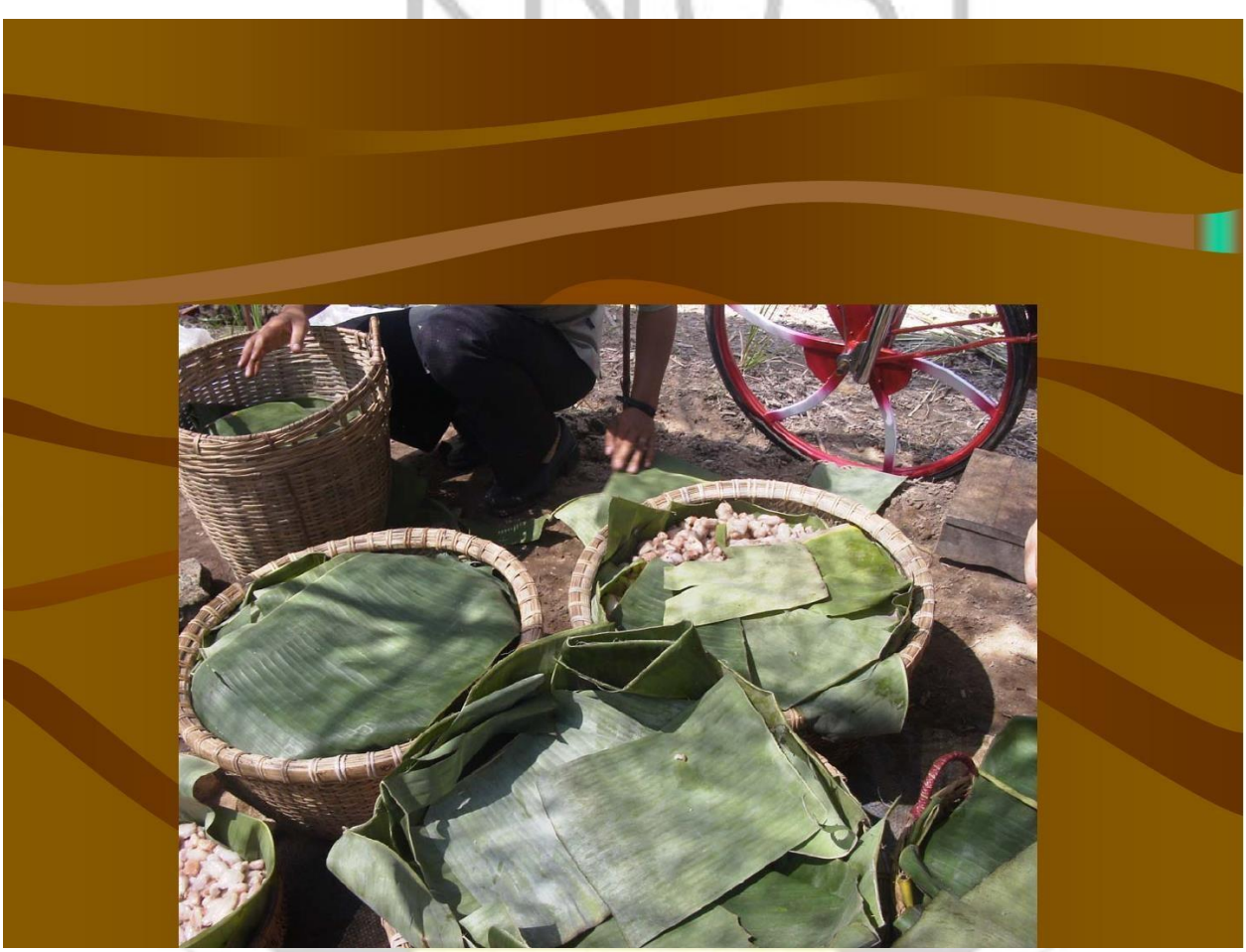

Plate 2.4 Basket fermentation (//documents.tips/documents/cacao-fermentation.html

The box fermentation type is the commonest type of fermentation practiced in the world. It can ferment beans of quantities of 25kg and beyond. The box is normally constructed with timber of dimensions 15cm wide and 2.5cm thick. In the absence of this thickness, the box can still be

constructed with plywood however there is the need to insulate the outer part with polystyrene in order to retain the heat.

Plate 2.5. Box fermentation (Yara.com)

2.10.2 Biochemical process of fermentation

Fermentation breaks down the mucilaginous pulp that surrounds the bean in order to release the compound that initiates aroma production. The fermentation process begins with oxygen diffusion reduction within the seed mass undergoing fermentation, thus creating an anaerobic environment. This is followed by the consumption of organic acids and pulp sugars by lactic acid bacteria and yeast leading to the subsequent production of lactates and ethanol. After 48 hour duration, pectinolytic yeast causes the degradation of seed pulp and drains the fluid trapped in the parenchymatous pulp. This reaction gives rise to rapid aeration which promotes the growth of acetic acid bacteria. Lactate, acetate and ethanol in addition to heat produced as a result of the

fermentation process brings about essential biochemical changes responsible for the typical and unique chocolate flavor, aroma and colour (Pohlan and Diaz Perez, 2010).

2.11 Drying of cocoa beans

Drying of cocoa beans can be done in two ways; natural (sun) or artificial. The sun drying is the simplest form of drying beans. After fermentation the cocoa beans retain a moisture content of about 50% and drying reduces the moisture content to an average of 7%. The final retention moisture is essential because a very high moisture content can promote mold growth. During drying, movement of air brings about moisture reduction. In the course of drying cocoa beans, certain reactions that promote the production of the natural cocoa flavor also takes place. Normally, drying takes place within a period of five to seven days during which there is evaporation of acids in the cocoa resulting in the final produce of low acid and highly flavoured cocoa bean. Drying cocoa beans to exceed seven days makes the cocoa beans susceptible to mold contamination (AusAid, 2010). Cocoa beans being dries are raked in order to allow uniformity in drying and break down agglomerate formation.

Sun drying is the usual mode of drying by small scale farmers. Cocoa beans are spread unto mats, polypropylene sheets etc . Under a good sunny weather, the cocoa beans are well dried within a week. During rainy seasons, drying of beans can be prolonged and cocoa beans also become susceptible to mold growth.

Artificial drying is the drying method mostly used by large scale farmers. In this method of drying, artificial dryers that use either wood or oil as fuel are used to dry the fermented cocoa beans. Cocoa beans can be heated directly by flue gases, however preferably, indirect heating using heat

exchangers is recommended. Drying should occur at a maximum temperature of 60C with duration not exceeding 48 hours. Drying under optimum conditions inhibits mold growth as well as enhancing biochemical oxidative reactions and loss of acids (Pohlan and Diaz Perez, 2010).

Plate 2.6 Drying of fermented cocoa beans (IFAD, 2012)

2.12 Storage of cocoa beans

Dried cocoa beans are bagged in jute bags, stored on pallets and stacked in a manner so as to avoid direct contact with the floors and walls of the warehouse. The jute bags should be clean, sealed and be able to withstand storage and transport conditions as well as discouraging pest harborage. The warehouse used for storage should be well aerated, dry, clean and free from pests' harborage.

Warehousing location should be free from odoriferous environment and substances which could easily contaminate the cocoa beans. Regular inspections and possible maintenance of the warehouse facility is needed in order to prevent mold growth.

Cocoa beans in storage should not be under the direct exposure of sunlight or other heat sources in order to prevent water migration and temperature differentials. During storage of cocoa beans, attention should be given to ensure protection of stored cocoa beans against cross-contamination, de-gradation and re-wetting. Monitoring of the moisture content of the stored cocoa beans at intervals is also recommended to ensure that levels are kept below 8%. It is important to maintain humidity of the warehouse below 70% RH (CAC/RCP 72-2013).

Plate. 2.7 Storage of cocoa beans in warehouse. Workers checking bags of cocoa beans stored in a warehouse in Abidjan (2015). (Photo credit: ISSOUF SANOGO/AFP/Getty Images)

In recent times, hermetic storage has been identified to protect cocoa beans during storage and transit. In this type of storage, a modified atmosphere is created which provides safe storage for many months. Hermetic storage naturally generates a low oxygen and high carbon dioxide

environment while maintaining a constant moisture content of the cocoa beans irrespective of the external environment. Since mold also require oxygen and high humidity levels for growth, hermetic storage discourages mold growth as well (Villers *et al.*, 2009)

Plate 2.8 SuperGrainbags-HC™

2.13 Transport of cocoa beans

During transportation of cocoa beans, measures are to be put in place to ensure protection of cocoa beans against possible mold contamination. Containers used for transport should be clean, well dried and without holes in order to prevent re-wetting of the cocoa beans which can make the cocoa beans susceptible to mold growth. The top layer and sides of cocoa bags stacked in the container for the transport should be lined with materials such as cardboard which can absorb condensate and provide protection against mold growth and subsequent ochratoxin contamination (CAC/RCP 72-2013).

2.14 Mycotoxins

A brief history about mycotoxins denotes the terminology was discovered in the year, 1962 when about 100,000 of turkeys died and the death was attributed to a probable contamination of secondary metabolites of *Aspergillus flavus* in a peanut meal (Forgacs, 1962).

Although mycotoxins are generally known to originate from fungi specie, the term mycotoxins does not cover all toxic compounds that emanate from fungal contamination. Molds could be beneficial or harmful. In addition to some molds being used for the fermentation of wine, wine and the production of cheese, salami; molds can also be used to produce antibiotics. Molds can be classified as mycotoxins or antibiotics as a result of their toxic or beneficial effects in treating diseases (Peraica *et al.*, 1999).

Toxins producing fungi are categorized into two groups; storage fungi and field fungi depending on their ecological needs for growth (Bankole, 1994). Storage fungi normally grow in foods having moisture content which is in equilibrium of 70%-80% of the relative humidity. The overall moisture content of the food is less than 18%. Penicillium and Aspergillus are important genera under this category. Although they are seldom present in field crops, they can be present in the environment, plant debris and surfaces giving that water activity is comparatively low. The field fungi are found in food with moisture content of exceeding 20%. They produce toxins before harvesting. Essential genera in this category are *Fusarium*, *Alternaria* and *Cladosporium*

Mycotoxins are generally hard to classify. They have diverse chemical structures with biosynthetic origins. They are also produced by a wide range of fungal species and have many different biological effects, hence scientists during categorization need expertise. For clinicians, they tend

to name and arrange them with respect to the organs the mycotoxins affect hence the names; hepatotoxins, neurotoxins, nephrotoxins etc. On the other hand, molecular cell biologist have resorted to naming them as teratogens, mutagens, etc as per their generic groups (Bennett and Kliche, 2003).

2.15 Exposure to Mycotoxins

Possible exposure to mycotoxins could be identified either through biological or environmental monitoring. There are two main sources of contamination my mycotoxins; one of the sources is the infection of the crop by mycotoxins producing molds while the crop is still on the farmland. The second source of contamination comes with the infection of the crop after harvesting, thus during storage. This kind of contamination is caused by storage mold and occurs when conditions of storage and drying are not optimal and can support the growth of mold. With regards to the storage conditions, humidity and temperature remain the most essential determining factors, with the moisture also playing a role in some agricultural produce (Eeckhout *et al.*, 2013).

Human exposure to mycotoxins could be identified through biological and environmental monitoring. Biological monitoring involves the direct assay of the presence of metabolites, adducts and residues in fluids, tissues and excreta. Environmental monitoring is carried out in food and air. Furthermore, biologically, mycotoxins could be identified by qualitative and quantitative measurements of residues and metabolites from fluids, tissues and excreta (Hsieh, 1988). Environmental monitoring involves measurements from air and food. Largely, one becomes exposed to mycotoxins by eating contaminated food. In addition, other exposures could be through dermal contact with surfaces contaminated with mold and also instances of inhaling spore-borne toxins (Fink-Gremmels, 1999). Mycotoxins exposure is commonly found in various locations of

the world where there are poor food handling and storage practices and also regulatory measures are weak (Barrett, 2000). Hence, the need for post-harvest guidelines to be put in place to prevent the contamination of ochratoxins and aflatoxins.

Ochratoxins contamination of cocoa beans may vary from one cocoa season to the other. The nature of the physical condition of the cocoa pods could also suggest possible contamination. Rotten pods, mutilated pods, mummified pods and those infested by insects relatively are more susceptible to ochratoxins contamination than healthy pods (Bastide *et al.*, 2006). Different climatic conditions in different growing areas can also determine the extent of contamination by ochratoxins.

2.16 Mycotoxicoses

Mycotoxicoses is the term for the resultant effect in the form of diseases acquired when humans and animals consume food contaminated by mycotoxins. These diseases occur through dietary, dermal, respiratory and other likely forms of exposure to toxic fungal metabolites. Toxicity of molds is classified into two namely acute and chronic toxicity with the acute form characterized by a quick response and the chronic form being a prolonged response of a relatively low dose exposure elapsing for a longer time duration which could possibly lead to cancer (James, 1985).

To identify a disease as mycotoxicoses, it is very important to ascertain a dose-responsive relationship between the mycotoxin and the disease in question and with regards to a targeted human population, epidemiological studies is required to establish the correlation. When during the studies, characteristic symptoms of the specific mycotoxicoses are reproducibly evoked by administering the mycotoxin under study, then substance evidence can be collected (Hsieh, 1988).

Mycotoxicoses is common to both developing and industrialized countries and occurs when social. Economic, environmental and meteorological (temperature and humidity) conditions together tend to promote the growth of mold (Peraica *et al.*, 1999).

The symptoms associated with mycotoxicoses are dependent on age, health status, amount and duration of exposure to toxins, dietary status, sex of the exposed person and type of mycotoxin exposed to. Mycotoxins poisoning could be severe with prevailing factors such as abuse of alcohol, vitamins and caloric deficiencies and also infectious diseases. Subsequently, mycotoxicoses tend to increase vulnerability to microbial diseases (Bennett and Klich, 2003).

Aflatoxicoses outbreaks have been reported in the tropics, mainly for adults in rural settings with poor nutritional status and having maize as their staple food. In these cases, there is acute toxic liver injury to affected persons (Tandon *et al.*, 1977). There seems to be a relationship between aflatoxins levels in blood of children and kwashiorkor. Studies in several tropical countries showed aflatoxins in liver specimens of children suffering from kwashiorkor (De Vries *et al.*, 1990).

Ochratoxin A which is the most toxic is known to be immunosuppressive, teratogenic, carcinogenic and nephrotoxic in all test animals that have been used for studies. In Tunisia, there has been the detection of ochratoxin A in the blood and food of patient having kidney impairment without known etiology (Maaroufi *et al.*, 1995a, 1995b). This assertion has also been found in food, feed and humans from other countries such as Bulgaria, Denmark, France, Italy, Hungary etc (Speijers *et al.*, 1993). Castegnaro *et al.*, (1990) suggested that ochratoxin A could possibly be the causal agent for urothelial tumours and endemic nephropathy.

2.17 Ochratoxins

Ochratoxin A primarily was discovered in 1965 as a metabolite of *Aspergillus ochraceus* during a research which was aimed as screening of fungal metabolites for the discovery of new emerging mycotoxins (Van der Merwe *et al.*, 1965). Later it was found in a trading corn sample in the US and identified as a potential nephrotoxic (Shotwell *et al.*, 1969). The amount of toxins produced is dependent on temperature, existence of competitive microfloria, moisture content the respective substrate of growth (Marquardt and Frohlich, 1992).

Studies have shown that *Aspergillus niger* aggregate and *Aspergillus carbonarius* are the main sources of Ochratoxin contamination in agricultural produce and products (Magnoli *et al*., 2007). It is found in many grains and several other kinds of food (Bayman and Baker 2006; Clark and Snedeker 2006). A review by Codex Alimentarius Commission in 2007 confirmed that the agricultural products that could be contaminated by ochratoxins A included cocoa. In addition to this, studies have shown the occurrence of ochratoxins in cocoa and cocoa products in a number of countries (Amezqueta *et al*., 2005). Ochratoxin A which is known to be mutagenic, teratogenic, immunosuppressive and genotoxic has been categorized by IARC as a Class 2B and possibly a carcinogen when exposed to humans (IARC, 1993). JECFA has confirmed PTWI of 100ng per kilogram body weight for Ochratoxin A.

Human exposure to ochratoxin has become common in recent times. Research has confirmed ochratoxin levels in the human serum and blood for the countries; Yugoslavia, Canada, Sweden and Western Germany (Kuiper-Goodman and Scott, 1989). Narrowing it down to Africa, urine analysis for children in Sierra Leone also confirmed the presence of ochratoxin and aflatoxin

(Jonsyn-Ellis, 2000)

Contamination of cocoa powder and chocolate by ochratoxin A have been found in 99.7% of samples (Burdaspal and Legarda, 2003). 81.3% of cocoa by-products of cocoa studies have shown were contaminated with OTA (Miraglia and Brera, 2002). Another study carried out by Tafuri *et al*, (2004) also indicated that OTA levels between the range of 0.22 and 0.77 µg/kg were found in ten cocoa powder samples sampled from the Italian market.

Research by Amezqueta *et al.*, (2005) has shown that ochratoxin contamination is concentration in the shells and the removal of shells could generally decrease the levels of ochratoxin by 6595%.

Generally, ochratoxins have been found to be metabolites of various species of Aspergillus namely *Aspergillus auricomus*, *Aspergillus melleus*, *Aspergillus carbonarius*, *Aspergillus glaucus*, *Aspergillus niger* and *Aspergillus alliaceus* (Abarca *et al.*, 1994; Bayman *et al.*, 2002; Cieglar *et al.*, 1972). They can also exist as metabolites of penicillium species. *Penicillium verrucosum* which produces OTA is a storage fungus, mainly thrives in relatively cool geographical locations such as North western Europe and proliferates at temperatures below 30C ^{o} (European Mycotoxin Awareness Network Fact Sheet 3). With regards to all toxins that are produced from the Aspergillus species, ochratoxin is the most potent. Ochratoxins could be present in coffee beans, oats, barley, rye, wheat and some other plant food (Marquardt and Frohlich, 1992). Unlike Penicillium species, Aspergillus species which thrive in high humidity and temperature areas grow in the sub-tropical and tropical regions. *Aspergillus carbonarius* shows a strong resistance to sunlight due to the existence of its black spores and therefore grows even in very high temperatures. Ochratoxins contamination also occurs in cocoa beans and cocoa products (Amezqueta *et al.*, 2005).

Ochratoxins which consists of derivative of isocoumarin are weak organic acids categorized as Ochratoxins A, B and C with distinct and unique chemical structures. They are different in terms of toxicity too with Ochratoxin A being the most potent and common. It can be present in food products even when there is no physical evidence of mold seen (Van der Merwe *et al.*, 1965).

In terms of stability, OTA has a moderate stability and is likely to survive most processes in the food chain ending up in consumer foods. It can only be partly reduced by food processes such as baking and roasting by just 20% (Puntaric *et al.*, 2001).

Primarily, ochratoxin A (L-phenylalanylcarbonyl-5-chloro-8-hydroxy-3,4-dihydro-3-*R*methylisocoumarin) (Turcotte *et al.*, 2013) affects the kidney hence it is nephrotoxin. It is known to be associated with Balkan endemic nephropathy (Krogh *et al.*, 1974). Animal studies have shown that it is toxic to the liver as well, a carcinogen, potent teratogen and also immunesuppresant (Beardall and Miller, 1994). OTA affects the physiology of the human cells in several ways and primarily, disturbs the enzymes responsible for the metabolism of phenylalanine by inhibiting the enzyme that synthesizes phenylalanine-Transfer-RNA Complex (Bunge *et al.*, 1979).

Following exposure to OTA commonly through consumption of food contaminated by OTA, it is absorbed from the gastro-intestinal tract. In several instances, Ochratoxin A absorption takes place from the stomach as a result of its characteristic acidic properties. After absorption, OTA is then transported by blood basically to the kidney and with lower concentrations of it being transported to the muscles and liver. This is associated with the metabolism of the proportion of OTA into non-toxic metabolite Ochratoxin alpha and to other less toxic metabolites in various parts of the body in different species (Galtier, 1978). OTA has a characteristic long serum half-life in humans and animals; 840 hours in humans and 72-120 hours in pigs. This can be attributed to its ability to bind strongly to serum macromolecules.

OTA contamination and subsequently levels of contamination vary from one cocoa season to the other. The physical conditions of the cocoa pods also determines the possible contamination of the cocoa beans by OTA. For instance, rotting pods, mutilated and insect infested pods are more susceptible to OTA contamination (Bastide *et al*., 2006).

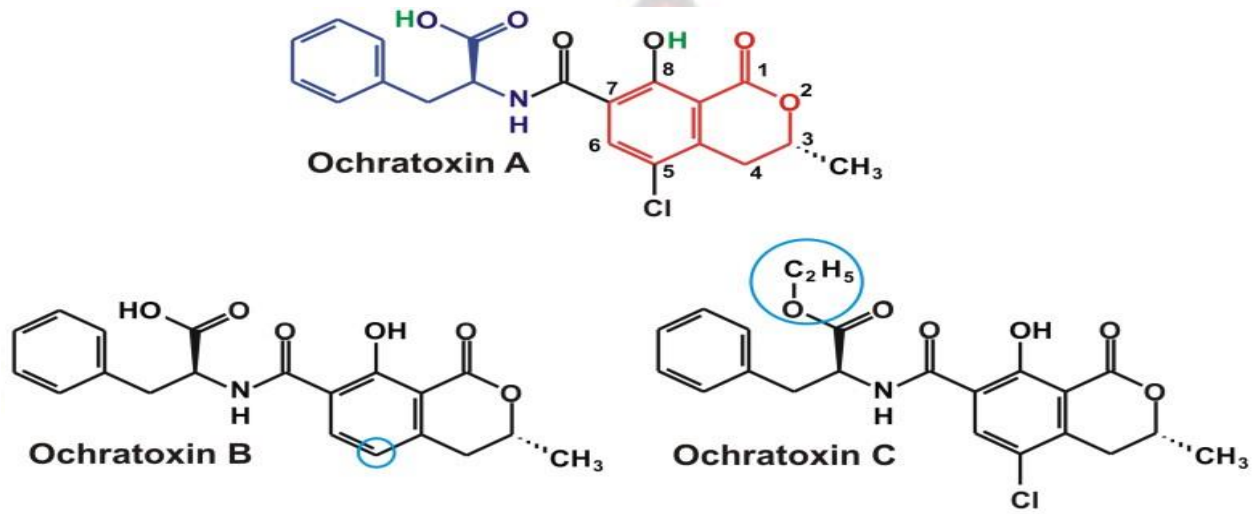

Fig. 2.1 Structure of Ochratoxins

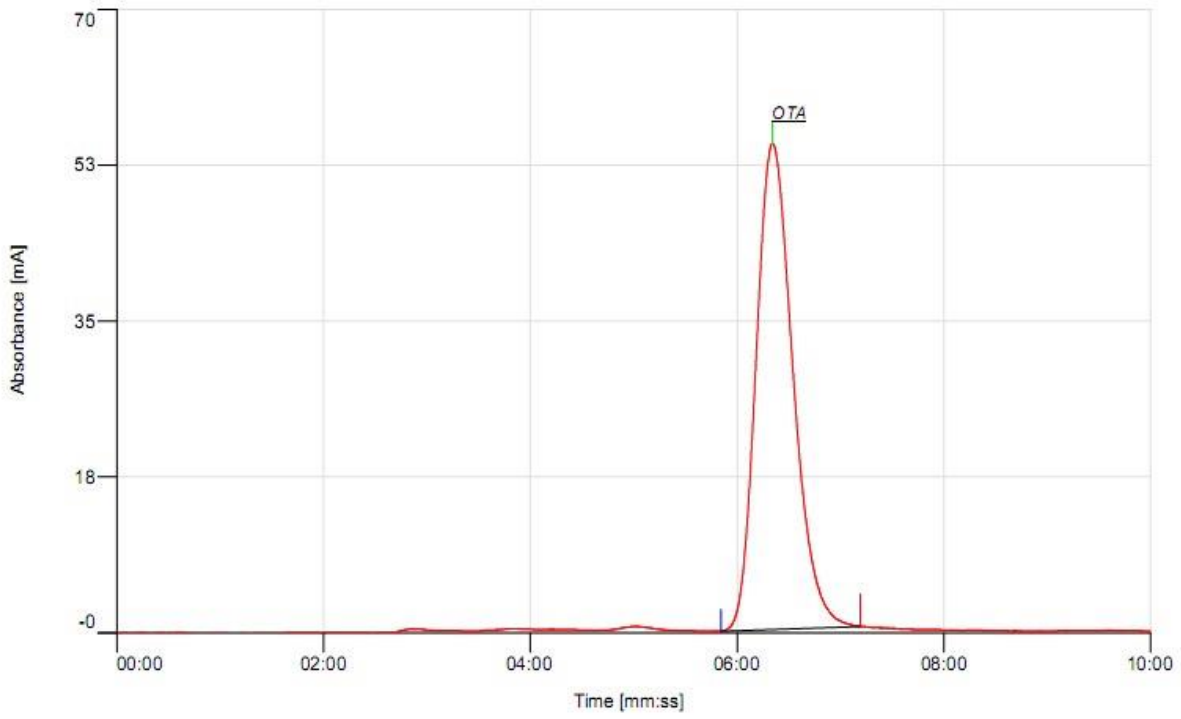

Fig 2.2 Chromatogram of OTA.

2.18 Aflatoxins

Aflatoxins which are produced by *Aspergillus flavus* and *Aspergillus parasiticus* are known to have carcinogenic, teratogenic, mutagenic and hepatoxic effects. There exist other Aspergillus species namely *Aspergillus nomius*, *Aspergillus pseudotamari* and *Aspergillus bombycis* which produce aflatoxins as well only that they are less common (Goto *et al.*, 1996). The different strains vary in their toxigenic propertities due to their varying levels of measurements qualitatively and quantitatively. Generally, crops could be contaminated with aflatoxins on the farm prior to harvest which is normally as a result of drought (Diener *et al.*, 1987). During storage, the moisture content and relative humidity of the environment can support mold growth (Detroy *et al.*, 1971, Wilson and Payne, 1994)). Naturally aflatoxins occur in the forms B1, B2, G1 and G2 with BI being the

most potent having been listed under the categorization of Class 1 carcinogen (IARC, 1993). Hydroxylation of Aflatoxin B_1 & B_2 results in the metabolites Aflatoxins M_1 & M_2 respectively. Medical tests using blood samples have shown a comparatively high proportions of West Africans are exposed to aflatoxins. Studies in this regard carried out in Nigeria, Gambia, Guinea Conakry and Senegal gave an estimation of 98% of subjects testing positive to aflatoxin markers (Wild, 1996). Presently lesser research studies have been made to determine the presence of aflatoxins in cocoa products as compared to ochratoxins in cocoa. Although it is important to note that information and data from the counties; Japan (Kumagai *et al*., 2008; Kawamura and Hamada 2009; Sugita-Konishi *et al*., 2010), Germany (Raters and Matissek 2000), Brazil (Copetti *et al*., 2011, 2012a, b) and Turkey (Dogan *et al*., 2006) indicate the presence of naturally occurring aflatoxins in cocoa and cocoa products. Studies have also shown the co-occurrence of aflatoxins and ochratoxins in cocoa (Tabata *et al*., 2008; Copetti *et al*., 2012a).

Aflatoxin contamination occurs either in the field or during storage between the temperatures of 20-40 ^{0}C, having 10-20% humidity and with 70-90% relative humidity in the air. The Aspergillus species normally grow at a temperature range of 8-55 ^{0} C with the optimal temperatures ranging from 36- ^{0} ^{0} 38 C and 25-35 C actively supporting aflatoxin production. Aflatoxins production is inhibited ^{0} ^{0} at temperatures below 10 C and above 45 C (Carvajal and Castillo, 2009).

Afs are derivatives of difuranocoumarin produced by polyketide pathway by mainly strains of *Apergillus parasiticus* and *Aspergillus flavus*. To a lesser extent other Aspergillus species also do produce Afs and these include *Aspergillus pseudotamari, Aspergillus bombycis,* and *Aspergillus nomius* (Goto *et al.*, 1996). Naturally, afs contamination can occur in several substrates and these include nuts, cereals, oilseeds, rice etc (Diener *et al.*, 1987).

Aflatoxins are converted to 8,9-epoxide, (catalyzed by the enzymes cytochrome P450) which has a potential to bind to proteins and DNA (Eaton and Groopman, 1994). The reactive epoxide can bind to guanine at the N^7 position. Aflatoxin B_1-DNA can also lead to GC to TA transversions. The presence of a reactive glutathione transferase system found in the microsomes and cytosol is responsible for the conjugation of activated aflatoxins (with reduced glutathione) resulting in the excretion of aflatoxins (Raj *et al.*, 1986). Aflatoxins susceptibility in various species is thus dependent on the levels of cytochrome P450 and glutathione transferase systems respectively (Eaton and Groopman, 1994).

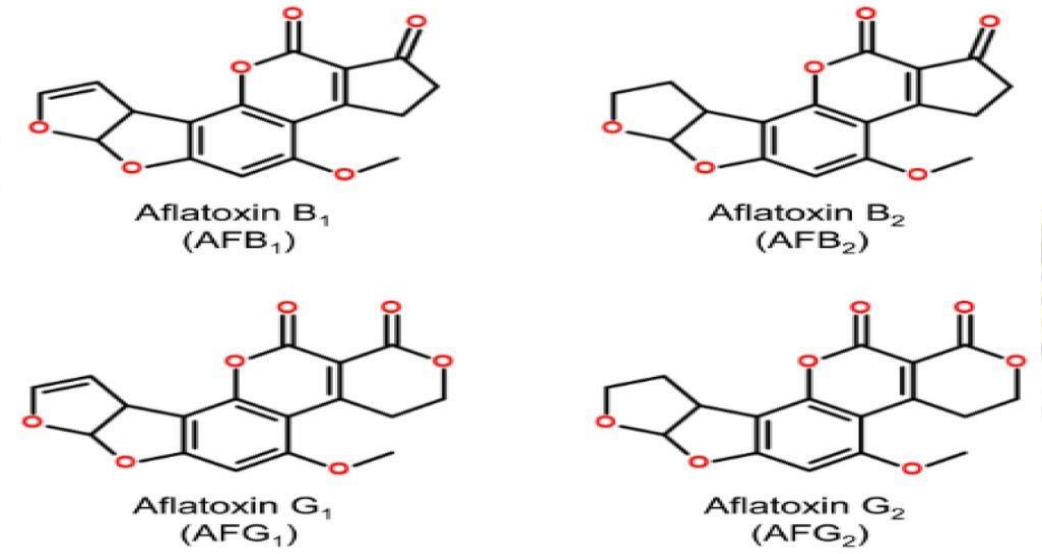

Fig. 2.3 Structure Aflatoxins B_1, B_2, G_1 & G_2.

2.19 Quality Control Measures for Management and Prevention of Mycotoxins

(Ochratoxins and Aflatoxins)

Ochratoxins and aflatoxins contamination greatly affects public health negatively and also overall agricultural output hence it is very essential to implement control measures for minimizing and preventing their contamination of food crop throughout the food chain. Cocoa after harvesting goes through sequential on-farm processes before it can be sold on the market. Harvesting done early enough is able to reduce the risk of aflatoxins contamination (Amyot, 1983).

The fermentation, drying and storage conditions of cocoa beans greatly contribute to it quality. The farmer's inability to perform these actions in compliance with required guidelines could later lead to deterioration either by disease and pest harborage or by mycotoxins producing molds. Cultivation, handling and marketing conditions also affect the quality of cocoa beans.

During fermentation, temperature, pH and a variety of microorganisms are involved. The huge numbers of microorganisms can include those capable of producing ochratoxins and aflatoxins. Studies by Mounjouenpou *et al*., (2011) revealed a huge proliferation if filamentous fungi as a result of the sweet mucilage.

Rapid drying of agricultural commodities to obtain low moisture content does not support growth of molds because of unavailability of water activity to promote their growth (Hamilton, 2000). After drying has achieved required moisture content, measures should be put in place to prevent cocoa beans from absorbing moisture from leaking roofs or even through condensation due to poor ventilation.

During transport and storage of cocoa beans, a rapid change and the magnitude of change in the humidity and temperature of the environment could pose the risk of fungal contamination. The storage of cocoa relative to other food crops is lengthy due to its prolonged shelf-life however,

longer storage duration increases the vulnerability to deterioration (Mabbett, 2013). The present temperature during storage contributes greatly to mold growth and possible mycotoxin contamination. When cocoa beans are stored in warehouse depending on the stack arrangement, there could be variations in the temperatures of cocoa beans within the stack and those outside, wet spots and condensation which may occur promotes mold growth. As a result of the presence of mold, temperatures at the contaminated regions will relatively increase to about 2-3C. It is therefore necessary to record temperatures at different locations and positions in the warehouse during storage. Proper ventilation becomes expedient in order to maintain uniformity in temperature and subsequently prevent mold contamination (Eeckhout *et al.*, 2013). Other factors that increase the susceptibility of cocoa to mycotoxins contamination include late harvesting, poor storage practices and lack of proper awareness (Bankole and Adebanjo, 2003).

Cocoa beans have a very high sensitivity towards relative humidity due to their hygroscopic property. In view of their ability to quickly absorb water from the air, for safe storage which would prevent mold growth it is necessary for the cocoa bean to have a maximum moisture content of 8% w/w (Mabbett, 2013).

Cleaning which forms of essential aspect of GMP is needed to get rid of dirt, dust and cocoa debris which tend to support mold growth. Cleaning equipment should also be maintained in a hygienic condition. Waste collection in the warehouse should be done more frequently. Pest control systems should be properly implemented as well (Eeckhout *et al.*, 2013).

The destruction of mycotoxins are very difficult and it becomes more efficient to prevent contamination of cocoa beans by ochratoxins and aflatoxins by controlling the exposure of the cocoa beans to molds responsible for producing these mycotoxins right from the beginning of the food chain to the end (thus from the farm to the final consumer). Implementation of HACCP will

allow various stakeholders along the food chain identify specific areas where ochratoxins/aflatoxins contamination is likely to occur and put in control measures to either prevent or minimize these hazards to acceptable levels (Mabbett, 2013).

Ochratoxin A molecule is known to have a strong resistance to very high temperatures administered during food processing (MAFF-Ministry Of Agriculture and Food, 1996). Ochratoxin A is concentrated in the shells portion of cocoa beans and relatively lower concentrations are in the nibs (Amezqueta *et al*., 2005, Manda *et al*., 2009). Thus, manually deshelling cocoa beans with hands is able to reduce Ochratoxin A levels to about 50-100% (Amezqueta *et al*., 2005, Manda *et al*., 2009). Industrial deshelling during manufacturing process could also reduce Ochratoxin A levels by about 48% (Gilmour and Lindblom, 2008).

It is very obvious the harm caused by ochratoxins and aflatoxins is known by researchers and scientists. It is however important for farmers to be broadly educated on mycotoxins and workshops organized at cocoa growing villages in the districts levels in order to create awareness on mycotoxins contamination.

2.20 Detoxification of ochratoxin A and prevention of absorption of Ochratoxin A

Elimination of contaminated commodities or the inactivation of mycotoxins present in these commodities leads to ochratoxin A detoxification (Kabak *et al.*, 2006). Measures to decontaminate OTA have been classified into physical, chemical and biological (Amezqueta *et al.*, 2009).

Physical measures include segregation or sorting, cleaning peeling and shelling, with the sole aim of removing contaminated portions of the food. Sorbents may also be used as nutritional additives to absorb OTA hence leading to the reduction in bioavailability of OTA. Chemically, compounds

such as bisulphites, ozone and ammonium can be used to destroy OTA (Riley and Norred, 1999). Biologically, some microorganisms can be utilized to detoxify OTA. Detoxification by microorganism is achieved through adsorption, transformation or decomposition (Karlovsky, 1999). Protozoa, bacteria, yeast and filamentous fungi are microorganisms responsible for the degradation of OTA. Microorganisms present in the rumen (mainly protozoa) hydrolyses OTA into Ochratoxin α which is of a lesser toxicity (Ozpinar *et al*., 1999). Petchkongkaew *et al*. 2008, also showed that isolation of *Bacillus licheniformis* from soyabean led to the elimination of 92.5% of OTA. *Lactobacillus acidophilus* VM 20 when used also showed a decrease in ≤ 95% OTA. Studies have also shown reduction in OTA by Lactobacillus acidophilus in yoghurt (Skrinjar *et al.*, 2002).

Filamentous fungi and yeast are also able to cause degradation of OTA. *Aureobasidium pullulans* was able to degrade OTA in grape must (Felice *et al*., 2008). *Phaffia rhodozyma* was also able to degrade OTA by more than 90% (Peteri *et al.*, 2007).

2.21 Detoxification of Aflatoxins

Similarly, aflatoxins could be detoxified by physical, chemical and biological means. However, physical and chemical means of detoxification are relatively disadvantageous in the sense that it doesn't result in the efficient removal of aflatoxin. Also there could be nutritional losses (Line and Brackett, 1995). Physically, detoxification can be performed through physical separation. Physical detoxification is less expensive and can be achieved best with small portions of the seeds being contaminated and also contaminated seeds showing distinct features (Brekke *et al.*, 1975). Chemically, ammonia can be used for detoxification. It is quite affordable and a familiar chemical

to the farmers. The effectiveness of ammonia usage for the purpose of detoxification depends on temperature, moisture and ammonia levels. In a research carried out, with moisture content of 15% and ammonia levels of 0.5%, initial aflatoxin concentration of 600µg/kg was decreased to concentration less than 20µg/kg within a period of three weeks at a temperature of 25C and similar reduction in three days at a temperature of 38C. (Brekke *et al.*, 1977). However, it is important to note that chemical detoxification is hazardous as associated with the usage of most chemicals (Bagley, 1979). Biological methods of aflatoxin detoxification involves the use of microorganisms such as bacteria, yeast and filamentous fungi. Their mode of action is on their ability to compete for interactions, nutrients and space (Fazeli *et al.*, 2009). Comparatively, biodegradation of aflatoxins by microorganisms as a means of aflatoxins removal becomes a better alternative because in addition to preserving the quality and safety of food (Alberts *et al.*, 2009), it has proven to be specific, cost effective, environmental friendly and efficient (Wu *et al.*, 2009).

Studies have shown that lactic acid bacteria are able to inhibit biosynthesis of aflatoxins or cause it removal, thus reducing its effects. Due to the fact that they cause fermentation as well, they could be used as starter culture for the production of food and drinks so they provide a dual benefit of fermentation as well as decontamination of mycotoxins (Shetty and Jesperson, 2006). Addition of aflatoxin B_1 to acidified milk and yoghurt (pH4.0) in concentrations of between 1000 to 1400g/kg resulted in about 90-97.8% reduction in Aflatoxin B_1 (Rasic *et al.*, 1991).

Research has also shown that the yeast; *Saccharomyces cerevisiae* is able to eliminate aflatoxins from contaminated media. At a temperature of ^{o} 37C, pH 3.0 with a contact time of 15 minutes, SC products (such as cell wall from baker or brewer yeast) caused AFB_1 elimination of about 2.5 to

49.3% with respect to the type of yeast product present as well as the concentration of toxins (Joannis-Cassan *et al.*, 2011).

2.22 Legislation of Ochratoxins and Aflatoxins

To completely eliminate natural toxicants from food is practically unachievable, hence they have a regulation which is quite different from food additives (FAO, 1997). Many government agencies all over the world including the European Union, the Institute of Public health in Japan and the U.S. Food and Drug Administration analyse products for ochratoxins, aflatoxins and other mycotoxins and therefore have established guidelines with respect to recommended safe doses. However, there is the need for an internationally harmonised mycotoxoins regulation. This is because the EU uses one set of guidelines, the U.S. FDA also uses another set and same goes for government agencies from other countries (Wilson *et al.*, 2002).

Based on analysis and evaluations carried out in the years; 1990, 1995 and 200, JECFA set provisional tolerable weekly intake (PTWI) of 100ng/kg bw/wk for OTA (JECFA, 2002).

The European Union 1881/2006 regulation determined maximum limits of ochratoxins in foods; 2µg/kg for wines and spices, 3µg/kg for cereal processed food products, 5µg/kg for cereals, 5µg/kg for roasted coffee and ground coffee, 5µg/kg for raisins and 10µg/kg for instant coffee.

In 2006, EFSA also came out to establish acceptable weekly intake of OTA to be 120ng/kg body weight and at the same time, TDI for aflatoxin was set at 1ng/kg body weight.

The EU restrained itself from establishing maximum acceptable limits of OTA in cocoa and cocoa products because high levels of OTA are seldom found in cocoa and cocoa products.

At the moment, there exists no Canadian standards with respect to maximum limits for OTA as well as aflatoxins in cocoa and cocoa products. Italy has established a limit of 0.05µg/kg for cocoa and derived products (FAO, 2003). Experts committee in the European Commision (EC) on the year, 2003 were proposing 2ng/g and 1ng/g OTA maximum limits in cocoa powder and chocolate respectively. Upon further considerations, the EC thought that based on these proposed maximum limits, it is not important to establish maximum limits for OTA in cocoa and other cocoa products. A survey in 2008 to 2009 showed that seven out of sixty cocoa products samples had OTA levels greater than the initially proposed EC maximum limits. Similarly, in 2011 to 2012, another survey conducted also showed that only two out of eighty cocoa products samples had OTA levels exceeding the initially proposed EC maximum limits giving a summary of nine out of one hundred and forty cocoa products samples. Mean levels are generally low but can however range from 0.07 to 7.8ng/g OTA levels (Turcotte *et al.*, 2013).

Some other countries have set regulations and limits with regards to ochratoxins and aflatoxins contamination for several varieties of food. Turkey has established limits within the range of 3.0 to 10µg/kg for food varieties. Switzerland has established limits of 5.0µg/kg for all food varieties with the exception of cereal based infant's foods which has a limit of 0.5µg/kg. Greece has ochratoxins limits of 20µg/kg for both raw and processed coffee. A limit of 15µg/kg is established for unroasted coffee in Hungary (FAO, 2003). Outside Europe, Uruguay has established a limit of 50µg/kg for cereals, rice and dried fruits (Lawley, 2013).

For aflatoxins, Finland has established a limits of 10 and 5µg/kg for all spices and other food products respectively. Germany also as a limit of 0.05µg/kg for foods meant for infants and young children. For Japan, AFB_1 established for all food is 10µg/kg. Russia has AFB_1 of 5.0µg/kg for

cocoa beans and cocoa products. In the United Kingdom, aflatoxins established limit for all food with the exception of milk is 20µg/kg (FAO, 2003).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Samples

Cocoa beans samples were obtained from QCC (Quality Control Company Division, COCOBOD) in Tema. Samples had been stored in freezers as recommended by WFLO commodity storage manual. Proper storage conditions is able to minimize insect infestation and inhibit mold growth.

Samples obtained were selected from districts in four cocoa growing regions namely Western South, Western North, Ashanti and Central regions.

For the analysis, sixty (60) samples were used comprising of twenty (20) from Western-South (ten unshelled and ten shelled), twenty (20) from Western-North (ten unshelled and ten shelled), ten (10) from Ashanti (five unshelled and five shelled) and ten (10) from Central (five unshelled and five shelled) regions.

Districts from Western-South region comprised of Wassa Akropong, Samreboi, Tarkwa, Bogoso, Prestea, Wassa Asikuma, Esiama, Asankragwa, Enchi and Dadieso.

Districts from Western North region comprised of Bibiani, Sefwi Wiawso, Manso Amenfi, Manso Adubia, Bonsu Nkwanta, Sefwi Bekwai, Bonsu Nkwanta (B), Sefwi Akontonbra and Bibiani.

Districts from Ashanti region comprised of Nsokote, Konongo, Juaso, Effiduase and Ampenim.

Districts from Central region comprised of Assin Breku, Assin Fosu, Twifo Praso, Breman Asikuma, Twifo Nyinase.

3.2 Methodology

3.3 Preparation of Ochratoxin Calibration standards

Calibration standards (0.5 ng/g, 1 ng/g, 2 ng/kg and 4 ng/g per 100ul injection volume) were prepared from 5ug/ml stock solution of Ochratoxin A. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluoresecence detetector (Ex: 333nm, Em: 477) with Phenonix C18, 5µm, 3.9 x 300 mm column was used. The mobile phase used was water: acetonitrile: acetic acid (49:49:2 v:v:v) at a flow rate of 1ml/min with column temperature maintained at 25°C. Limit of Detection and Limit of Quantification were established at 0.3 ng/g and 1 ng/g respectively.

3.4 Preparation of Aflatoxin calibration standard

Calibration curve of aflatoxin Mix (G_1, G_2, B_1, B_2) standards were prepared from Supelco® aflatoxin standard of 2.6 ng/µL in methanol. Calibration concentrations for B_1 and G_1 were 0.5, 1, 2, 8, 16 ng/g per 100ul injection while B_2 and G_2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100ul injection. Limit of Detection and Limit of Quantification for total aflatoxin was established at 0.5ng/g and 1ng/g respectively.

A Cecil-Adept® Binary Pump HPLC coupled with Shimadzu® 10AxL fluorescence detector (Ex: 360 nm, Em: 435 nm) with Phenomenex® HyperClone BDS C18 Column (150 x 4.60mm, 5 um) was used for aflatoxin analysis. The mobile phase used was methanol: water (40:60, v/v) solution at a flow rate of 1ml/min with column temperature maintained at 40°C. To 1 liter of mobile phase 119 mg of potassium bromide and 350ul of 4M nitric acid were added (required for postcolumn electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone®).

3.5 Analytical Procedure

Modified QuEChERS-HPLC method was used for the determination of aflatoxins and ochratoxins if present in the cocoa samples.

3.5.1 Extraction and Purification

Modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method (Sirhan *et al.*, 2014) was used for the extraction. Exactly 2g of thoroughly homogenized cocoa samples were weighed into polypropylene centrifuge (15ml). Then 3.0ml of 60:40 (%v/v) methanol/acetonitrile was added and vortex for 3 minutes. Afterwards, 1.32g of anhydrous $MgSO_4$ and 0.25g of NaCl were added to the mixture and vortex for 1 minute. The mixture is then centrifuged for 5 minutes at

4000rpm and the upper organic layer filtered through a 0.45µm nylon syringe or by means of ultrafiltration prior to injection. Afterwards, 10-100µl was injected into the HPLC. Recovery was at 93% at 10 ng/g spiked level.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Ochratoxin Analysis

4.1.1 Introduction

A total of sixty (60) cocoa samples were analysed for Ochratoxin A and Aflatoxins. A standard calibration of OTA is presented in Figure 4.1.

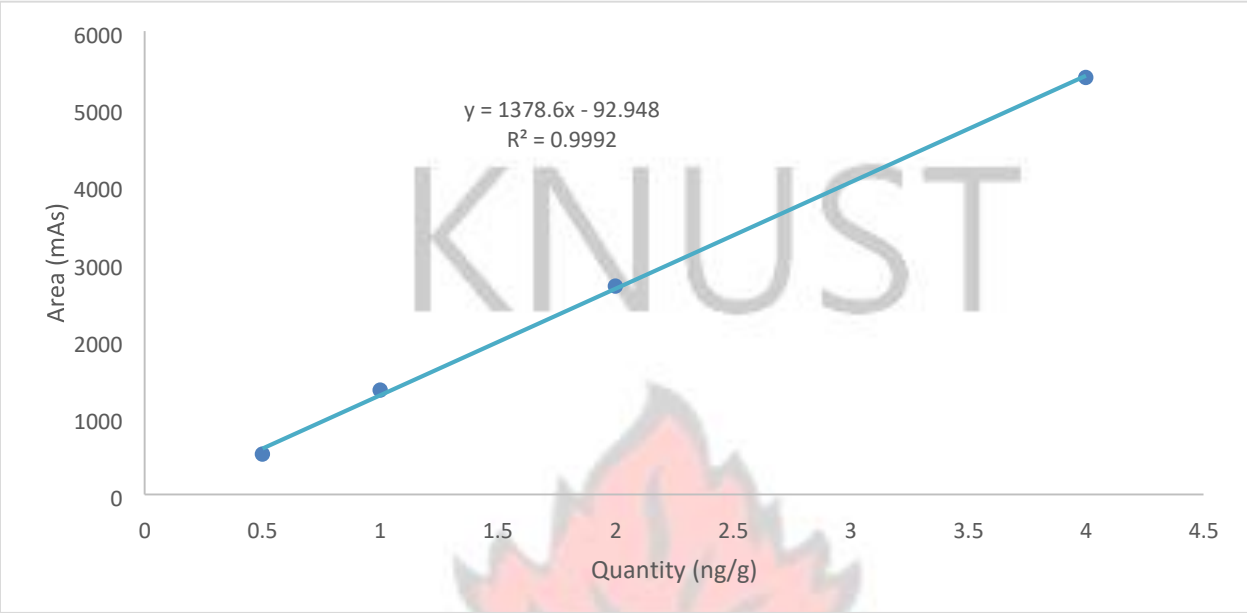

Figure 4.1 Typical standard calibration curve of OTA.

Table 4.1 Levels of OTA in cocoa beans sampled from the Western South.

Districts/Status	Concentration (ng/g) of OTA in cocoa beans	Districts/ Status	Concentration (ng/g) of OTA in cocoa beans
Wassa Akropong (S)	ND	Wassa Akropong (US)	ND
Samreboi (S)	ND	Samreboi (US)	ND
Tarkwa (S)	ND	Tarkwa (US)	ND
Bogoso (S)	ND	Bogoso (US)	ND
Prestea (S)	ND	Prestea (US)	ND

Wassa Asikuma (S)	ND	Wassa Asikuma (US)	ND
Esiama (S)	ND	Esiama (US)	ND
Asankragwa (S)	ND	Asankragwa (US)	ND
Enchi (S)	ND	Enchi (US)	ND
Dadieso (S)	ND	Dadieso (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

4.1.1.1 Samples from Western South region

Samples from ten districts selected from Western south region were analyzed with shells or without shells and for all samples OTA could not be detected as levels if present were extremely low.

Studies into OTA contamination of cocoa beans are quite few. Mounjouenpou *et al*., (2008) carried out OTA analysis on dried fermented cocoa beans. In their study, results showed that contamination of OTA in cocoa beans were generally low if the cocoa beans were gotten from intact pods, giving OTA levels within the range of non- detectable to and 0.27ng/g.

Table 4.2 Levels of OTA in cocoa beans sampled from the Western North.

Districts/Status	Concentration (ng/g) of OTA in cocoa beans	Districts/ Status	Concentration (ng/g) of OTA in cocoa beans
Bibiani A (S)	ND	Bibiani A (US)	ND
Sefwi Wiawso (S)	ND	Sefwi Wiawso (US)	ND
Manso Amenfi (S)	ND	Manso Amenfi (US)	ND
Manso Adubia (S)	ND	Manso Adubia (US)	ND
Bonsu Nkwanta (S)	ND	Bonsu Nkwanta (US)	ND

Sefwi Bekwai (S)	ND	Sefwi Bekwai (US)	ND
Bonsu Nkwanta B (S)	ND	Bonsu Nkwanta B (US)	ND
Sefwi Akontonbra (S)	ND	Sefwi Akontonbra (US)	ND
Sefwi Akontonbra B(S)	ND	Sefwi Akontonbra B(US)	ND
Bibiani B (S)	ND	Bibiani B (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

4.1.1.2 Samples from Western North region

Samples from ten districts selected from Western north region were analyzed with shells or without shells and for all samples OTA could not be detected as levels if present were extremely low.

Table 4.3 Levels of OTA in cocoa beans sampled from Ashanti

Districts/Status	Concentration (ng/g) of OTA in cocoa beans	Districts/ Status	Concentration (ng/g) of OTA in cocoa beans
Nsokote (S)	ND	Nsokote (US)	ND
Konongo (S)	ND	Konongo (US)	ND
Juaso (S)	ND	Juaso (US)	ND
Effiduase (S)	ND	Effiduase (US)	ND
Ampenim (S)	ND	Ampenim (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

4.1.1.3 Samples from Ashanti region

Samples from five districts selected from Ashanti region were analyzed with shells or without shells and for all samples OTA could not be detected as levels if present were extremely low **Table**

4.4 Levels of OTA in cocoa beans sampled from Central

Districts/Status	Concentration (rcocoa of OTA in beans	Districts/ Status	Concentration (ng/g) of OTA in cocoa beans
Assin Breku (S)	ND	Assin Breku (US)	ND
Assin Fosu (S)	ND	Assin Fosu (US)	ND
Twifo Praso (S)	ND	Twifo Praso (US)	ND
Breman Asikuma (S)	ND	Breman Asikuma (US)	ND
Twifo Nyinase (S)	ND	Twifo Nyinase (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

4.1.1.4 Samples from Central region

Samples from five districts selected from Central region were analyzed with shells or without shells and for all samples OTA could not be detected as levels if present were extremely low.

4.2 Aflatoxin Analysis

Standard calibration curves of Aflatoxins are presented in Figures 4.3 to 4.6 respectively.

Aflatoxin G1

Quantity [ng/g] = 0 + 3.01167 * Area [As] r^2=

0.998264

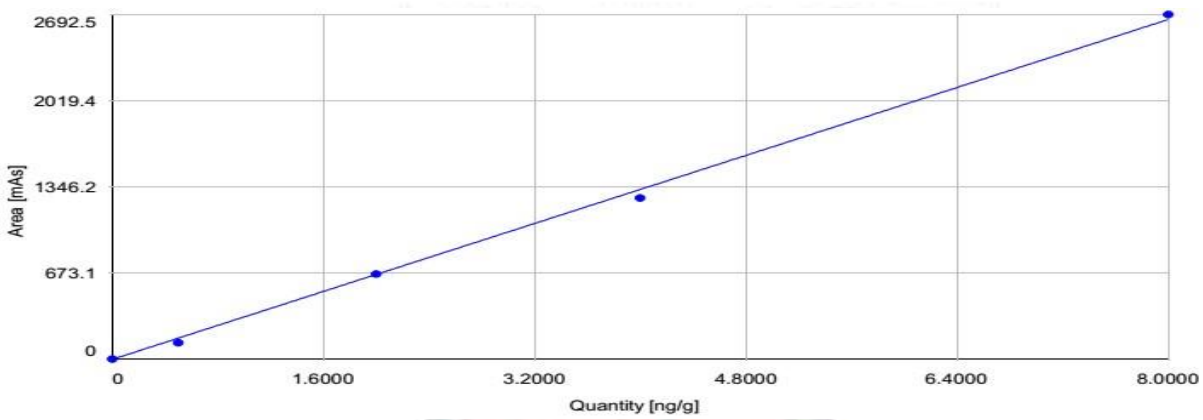

Fig 4.2 Standard calibration curve of Aflatoxin G1

Aflatoxin G2

Quantity [ng/g] = 0 + 1.84477 * Area [As]

r^2= 0.995416

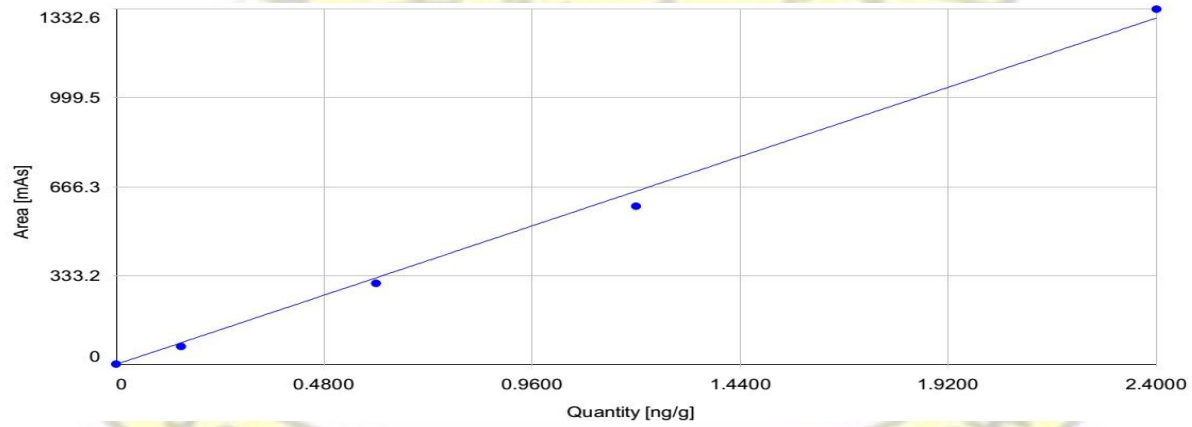

Fig 4.3 Standard calibration curve of Aflatoxin G2

Aflatoxin B2

Quantity [ng/g] = 0 + 0.724454 * Area [As]

r^2= 0.993736

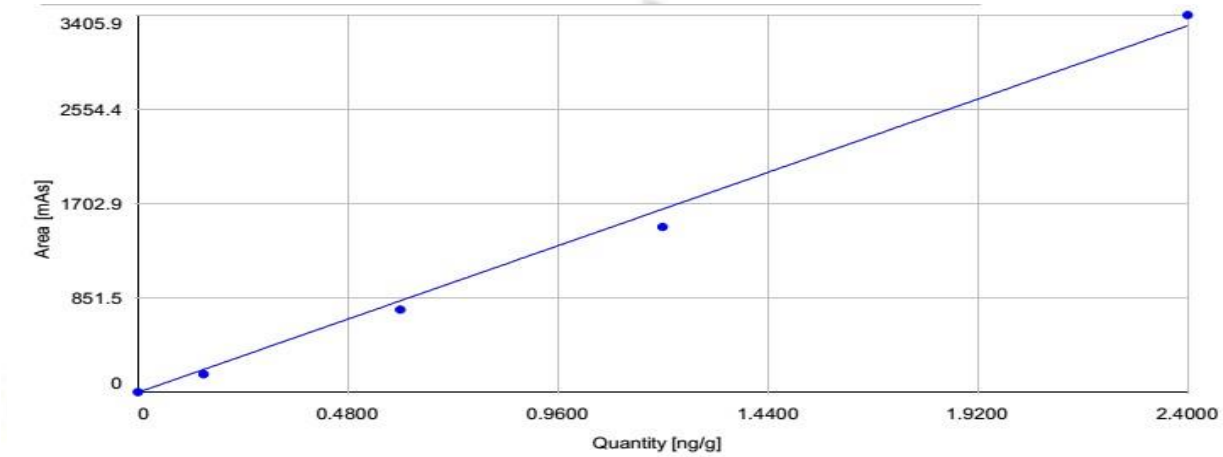

Fig 4.4 Standard calibration curve of Aflatoxin B2

Aflatoxin B1

Quantity [ng/g] = 0 + 1.00345 * Area [As]

r^2= 0.996376

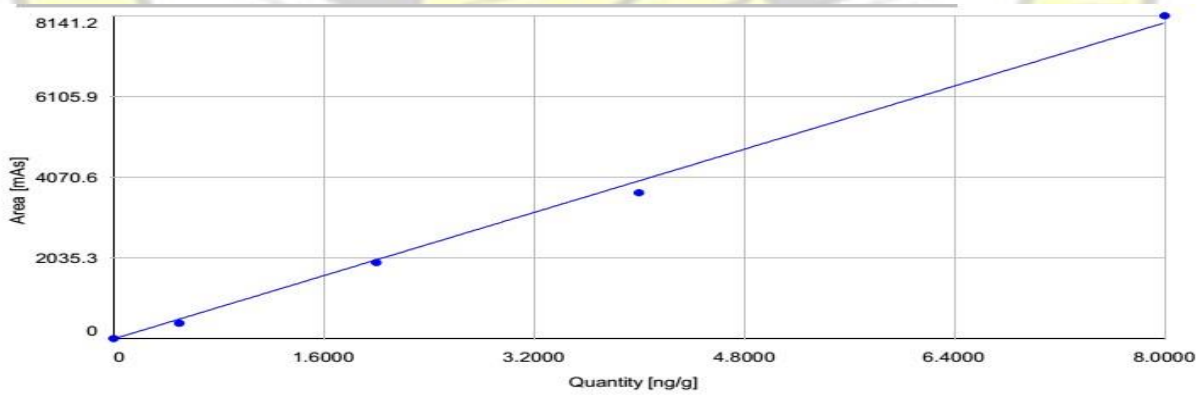

Fig 4.5 Standard calibration curve of Aflatoxin B1

Table 4.5 Levels of Aflatoxins in cocoa beans sampled from the Western South.

Districts/Status	Concentration (ng/g) of Aflatoxins in cocoa beans	Districts/ Status	Concentration (ng/g) of Aflatoxins in cocoa beans
Wassa Akropong (S)	ND	Wassa Akropong (US)	ND
Samreboi (S)	ND	Samreboi (US)	ND
Tarkwa (S)	ND	Tarkwa (US)	ND
Bogoso (S)	ND	Bogoso (US)	ND
Prestea (S)	ND	Prestea (US)	ND
Wassa Asikuma (S)	ND	Wassa Asikuma (US)	ND
Esiama (S)	ND	Esiama (US)	ND
Asankragwa (S)	ND	Asankragwa (US)	ND
Enchi (S)	ND	Enchi (US)	ND
Dadieso (S)	ND	Dadieso (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

Table 4.6 Levels of Aflatoxins in cocoa beans sampled from the Western North.

Districts/Status	Concentration (ng/in of Aflatoxins coc beans	Districts/ Status	Concentration (ng/in of Aflatoxins coc beans
Bibiani A (S)	ND	Bibiani A (US)	ND
Sefwi Wiawso (S)	ND	Sefwi Wiawso (US)	ND
Manso Amenfi (S)	ND	Manso Amenfi (US)	ND
Manso Adubia (S)	ND	Manso Adubia (US)	ND
Bonsu Nkwanta (S)	ND	Bonsu Nkwanta (US)	ND
Sefwi Bekwai (S)	ND	Sefwi Bekwai (US)	ND
Bonsu Nkwanta B (S)	ND	Bonsu Nkwanta B (US)	ND
Sefwi Akontonbra (S)	ND	Sefwi Akontonbra (US)	ND
Sefwi Akontonbra B(S)	ND	Sefwi Akontonbra B(US)	ND
Bibiani B (S)	ND	Bibiani B (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

Samples from ten districts selected from Western South and Western-North region were analyzed with shells or without shells and for all samples Aflatoxins could not be detected as levels if present were extremely low.

Studies carried out by Mounjouenpou *et al*., (2011) and Mabbett, (2013) suggests that proper fermentation, drying and storage of cocoa beans carried out by farmers within the harvesting season contributed to the inability to detect levels of OTA and aflatoxins in cocoa beans as the

ability of cocoa farmers to properly ferment, dry and store the cocoa beans as per the required and recommended practices leads to prevention or reduction in contamination by the fungi producing toxins.

Table 4.7 Levels of Aflatoxins in cocoa beans sampled from Ashanti

Districts/Status	Concentration (ng/g) of Aflatoxins in cocoa beans	Districts/ Status	Concentration (ng/g) of Aflatoxins in cocoa beans
Nsokote (S)	ND	Nsokote (US)	ND
Konongo (S)	ND	Konongo (US)	ND
Juaso (S)	ND	Juaso (US)	ND
Effiduase (S)	ND	Effiduase (US)	ND
Ampenim (S)	ND	Ampenim (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

Table 4.8 Levels of Aflatoxins in cocoa beans sampled from Central

Districts/Status	Concentration (ng/g) of Aflatoxins in cocoa beans	Districts/ Status	Concentration (ng/g) of Aflatoxins in cocoa beans
Assin Breku (S)	ND	Assin Breku (US)	ND
Assin Fosu (S)	ND	Assin Fosu (US)	ND
Twifo Praso (S)	ND	Twifo Praso (US)	ND
Breman Asikuma (S)	ND	Breman Asikuma (US)	ND
Twifo Nyinase (S)	ND	Twifo Nyinase (US)	ND

*S- Shelled *US- Unshelled *ND- Not Detected

4.2.1 Samples from Western South, Western North, Ashanti and Central regions

Samples from thirty districts selected from Western South, Western North, Ashanti and Central regions were analyzed with shells or without shells and for all samples Aflatoxins could not be detected as levels if present were extremely low and below the LOD.

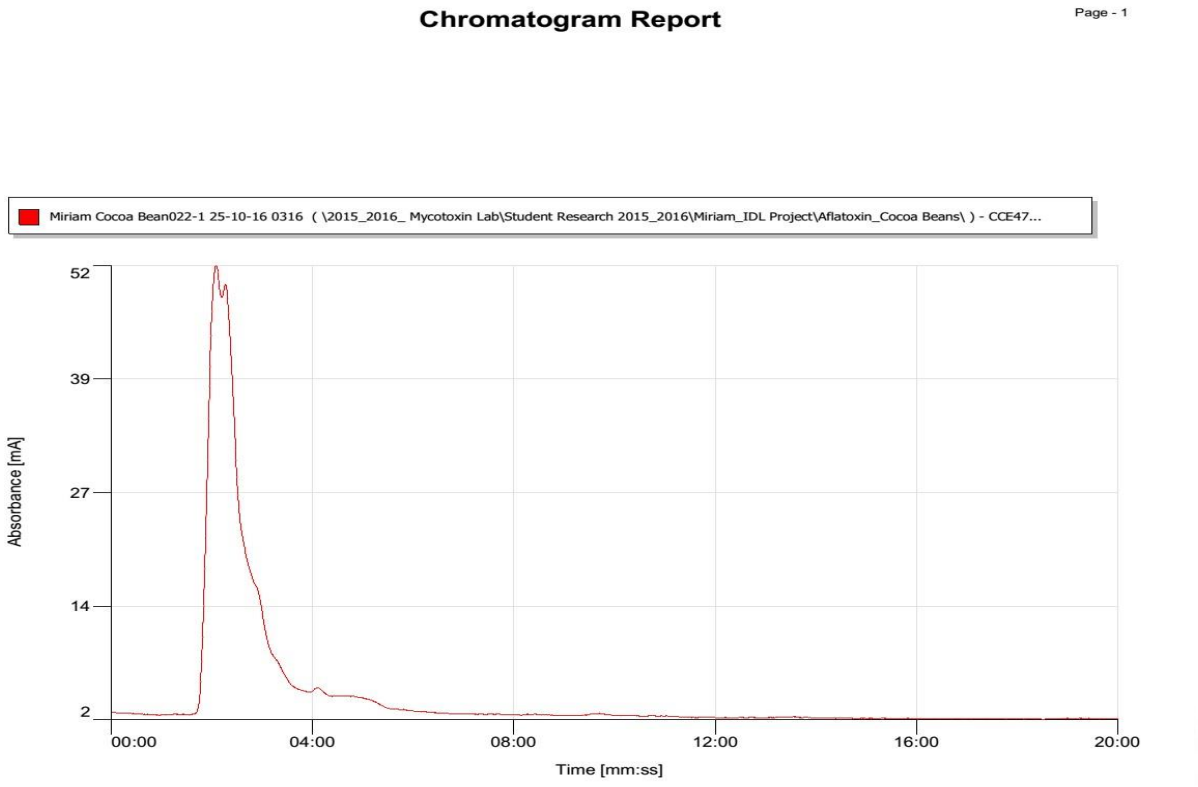

Fig 4.6 Chromatogram report of Aflatoxins analysis

In summary sixty samples were analyzed for OTA and aflatoxins respectively with thirty samples of cocoa beans shelled and thirty samples of cocoa beans unshelled. Studies carried out by Amezqueta *et al.*, (2005) showed that manual shelling could cause OTA loss within the range of 95% to 50% suggesting that OTA contamination could be concentrated in the shells. Hence the analysis took into consideration the impact of shelling and otherwise. Gilmour and Lindblom (2008) in their study also showed that industrial shelling could lead to a reductions in OTA contamination by 48%.

A study carried out by Copetti *et al.*, (2013) to determine the occurrence of OTA in cocoa by products and its possible reduction during chocolate manufacture found out that although they

recorded contamination of cocoa products by OTA, the contamination levels were generally low. The cocoa shells had OTA concentration levels within the range of 0.13 to 2.10µg/ kg and the cocoa nibs recorded OTA levels of <LOD to 0.38µg/kg. Per this study, contamination levels in shells were about ten times that found in nibs, affirming that contamination levels could be concentrated in the shells relative to the nibs. The shelling stage in the cocoa processing technology is therefore very important due to the fact that it leads to reduction in contamination by mycotoxins including ochratoxin A. Ministry of Agriculture, Fisheries and Food (MAFF, 1996) confirmed that the molecule of Ochratoxin A is able to resist most heat treatment administered to it during food production.

For co-occurrence of OTA contamination and aflatoxins contamination, a similar study carried out by Turcotte *et al.,* (2013), to assess the analysis of cocoa products for OTA and aflatoxins did not show a specific correlation with these two forms of contamination because results from their study indicated that samples highly contaminated with OTA did not show similar high levels of aflatoxins contamination. At the end of their 2011-2012 survey, using 80 samples, only 2 out of 80 samples (excluding cocoa butter) recorded OTA levels greater than previously proposed EC limits for OTA contamination (Expert committee of the EC was considering in 2003 to set maximum OTA contamination levels at 1ng/g and 2ng/g in chocolate and cocoa powder respectively). Copetti *et al*., (2012) on the other hand in their study showed the co-occurrence of OTA and Aflatoxins B in chocolate found on the markets of Brazil. After their research, the mean concentrations of OTA and Aflatoxins B_1 reported in powdered raw chocolate were 0.39 and 0.43ng/g respectively and that for dark chocolate were 0.34 and 0.43 ng/g respectively.

In a study carried out in Ivory Coast to determine OTA levels in cocoa beans of different grades harvested within the periods of 2005 and 2009, a total of 1895 samples were analyzed. The study

considered quantification of OTA with respect to exportable beans, non-exportable beans and the total beans throughout the harvesting seasons. Exportable beans recorded the lowest level of contamination ranging between 0.64±0.53 µg/kg. The percentage of beans below LOD limit of 0.05 µg/kg was 18%. Hence exportable beans from Ivory Coast with respect to the result from the study all had levels below the 2µg/kg maximum limit as proposed by European Communities (CA, 2207) regulations. For shelled beans the results ranged from 0.19±0.16 (Coulibaly *et al*., 2013).

The extremely low levels of OTA and aflatoxins in cocoa beans harvested from Western-South, Western-North, Ashanti and Eastern regions are commendable especially because these are exportable beans hence were of good quality for the market.

Although some of the beans were moldy, mold contamination could not produce mycotoxins. Temperature and water activity are factors that contribute to the growth of fungi and possibly the formation of mycotoxins (Magan *et al.*, 2011). Generally, temperature range of 20ºC-30ºC is optimum for the production of mycotoxins by molds and the interaction between optimal temperatures with water activity is essential for the growth of mycotoxins (Medina *et al.*, 2013). Therefore the inability to detect OTA and aflatoxins in the beans sampled from Western-South, Western-North, Ashanti and Central regions can be attributed to the fact that during fermentation, drying and storage, these factors necessary for mycotoxin formation were not present in sufficient proportions to cause contamination by mycotoxins particularly OTA and aflatoxins. Quality measures are expedient in cocoa harvesting and in all the stages prior to cocoa processing due to the fact that mycotoxins could resist temperature treatment to an extent.

Again, the extremely low levels are below the proposed 2µg/kg limit by the European Communities (Codex Alimentarius, 2007), suggesting that adherence to GAP with respect to cocoa beans production will promote large volumes of exports to the European markets.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results from the study indicated that out of the sixty samples (60) analyzed from the four regions, namely; Western-South, Western-North, Ashanti and Central regions, no OTA or aflatoxins were detected under the experimental conditions. The samples therefore satisfy the proposed limit of 2 µg/kg set by the European Commission.

5.2 Recommendations

- For future studies, beans from other districts in cocoa growing regions can be used to assess the overall susceptibility of cocoa beans cultivated in Ghana to mycotoxins contamination.
- The entire process steps from cultivation to storage and transport should be properly documented for adaptation of proper measures which will prevent contamination of cocoa beans by mycotoxins.

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