KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

SYNTHESIS AND INTERVENTIVE PERFORMANCES OF ALUMINA, SILICA AND THEIR COMPLEXES IN AFLATOXIN ADSORPTION *IN-VITRO*

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 (Food Quality Management)

By

ILOABUCHI CHINWENWA GOLD

B.Tech Food Sci. and Tech

March 2016

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CERTIFICATION

I hereby declare that this thesis is the result of my own work and to the best of my knowledge contains no material previously published by another person or submitted for the award of degree in any other university except where acknowledgement has been accurately made in the text.

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ABSTRACT

Aflatoxin contamination of foods is a major concern in the world especially in developing countries. Various binding agents have been used to deactivate aflatoxins in food. In this study, an experimental design using response surface methodology (RSM) was applied to develop a model in order to predict aflatoxin adsorption potential of alumina and silica complexes (ASC) *in- vitro*. The independent variables were amount of alumina (A: 20 to 60 %), silica (B: 40 to 80 %), heating time (0 to 4 h) and heating temperature (0 to 1400 ^{o}C) and the dependent variable was reduction in total aflatoxin. Design Expert was used in a combined D-optimal, quadratic x quadratic mixed process design to generate 28 runs for the experiment. The various complexes were screened against a stock solution of naturally contaminated peanuts with aflatoxin content of 456.3ppb in order to ascertain the adsorption capacity of the different alumina-silica complexes. The ANOVA and model fit test showed that aflatoxin adsorption by ASC is significant. All the non-heated samples of ASC showed percentage reduction of aflatoxin above 70 %. The micro graphical analysis of the samples of ASC also revealed the grains as having different size ranging from 154 µm to 490 µm. The predicted optimum conditions for the aflatoxin adsorption of ASC are combination of alumina and silica at a ratio of 32Al:68Si, heated at a temperature of 350 ^{o}C for 3 h.

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ABBREVIATIONS

AfB1	Aflatoxin B1
ASC	Alumina-silica complexes
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic acid
EFSA	European Food Standard Agency
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
PCB	Poly chlorinated biphenyls
Rpm	Revolutions per minute
TAfT	Total aflatoxin µg/kg

microgram per kilogram **TABLE OF CONTENTS**

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

1.1.1 Aflatoxins

Aflatoxins are toxic metabolites produced by two species of the genus *Aspergillus flavus* and *Aspergillus parasiticus*. Among the aflatoxins that can be found (B1, B2, G1, G2, M1 and M2), the most important is aflatoxin B1, because it is the most prevalent in foods and toxic for humans and animals. Aflatoxins started getting attention in 1960 when 100,000 turkeys and other poultry in the UK died in a single event. Cause of death was eventually traced to a toxic contaminant in groundnut meal used in the bird's feed which was later named aflatoxin (Lawley, 2013). Since then, a lot of discoveries have been made determining the different types of aflatoxins in existence, the type of foods that are easily affected the resulting diseases due to consumption at a certain quantity and so on.

According to Richard (2007), aflatoxins are primarily hepatotoxic; however, susceptibility varies by breed, species, age, length of exposure and nutritional status of the animal. Depending on dose and duration of exposure, aflatoxicosis which is a disease caused by aflatoxins could lead to acute illness and death, nutritional and immunological consequences and cancer. The steady ingestion of aflatoxin contamination foods at low levels but over a long period of time has been found to result in jaundice, primary liver, cancer cirrhosis chronic hepatitis, and impaired nutrient conversion (Lawley, 2013).

1.1.2 Consumption of Foods Susceptible to Aflatoxin

Aflatoxins may be present in a wide range of food commodities, especially cereals, tree nuts, oilseeds, and spices. Although aflatoxins have been detected in many other food commodities,

maize, groundnuts (peanuts), pistachios, chilies, black pepper, dried fruit and figs are all known to be high risk foods for aflatoxin contamination (Lawley, 2013). A study by Bandyopadhyay *et al.* (2007) shows that maize is more likely to be contaminated with aflatoxins and contaminated at a higher level than sorghum or millet. In Ghana, maize is one of the major staple foods consumed widely and it is also the third most important crop in the world next to rice and wheat. However, peanuts also form a significant part of the Ghanaian diet. Nigerians also, on the average consume 138 kg of cereals annually of which the primary cereal is corn (Bandyopadhyay *et al.*, 2007).

Foods susceptible to aflatoxin are seen to form the basic diets of Ghanaians and people in many other African countries. It is also common knowledge that most times, even visibly damaged foods are not discarded. The people rather look for means of making the contaminated foods look better (maybe by washing or cooking for a longer period), after which they consume. Some of the farmers of these crops keep the damaged crops for their family and sell the whole ones for higher prices. According to the report of Masters *et al.* (2013), nearly two million of Ghanaians remain vulnerable to food insecurity. The issue of food insecurity is still prevalent in many countries especially in the rural areas hence the possible consumption of contaminated foods.

Aflatoxin contamination causes huge losses to farmers. In Ghana 5-15 % peanuts are thrown away after sorting (Awuah *et al.*, 2006). This is due to the fact that once the peanuts are contaminated; it is very visible and not pleasant to the eyes. Any compromise to the wholesomeness of peanut shells whether by insect infestation or by handling only encourage contamination by these toxins (Masters *et al.*, 2013).

1.1.3 Binders

Over the years, efforts are being made to curb aflatoxin contamination of foods and feeds. Adsorption reduction using certain binding agents has been named as the best known method for mycotoxin deactivation (Murugesan *et al.,* 2015). These binders can also be called adsorbents or enterosorbents and they can be of organic (microbial) or inorganic (e.g. clay) nature. Some known binders include activated carbon, alumino-silicate binders such as zeolite, bentonite, montmorillonite, yeasts, lactic acid bacteria and organic polymers such as cellulose etc.

Most of the additives and adsorbents capable of adsorbing aflatoxins cannot be wholly trusted to be safe for the body. They could in themselves be toxic, bind essential nutrients in the food or cause some other negative effects in the body. Therefore *in-vitro* studies and series of *in-vivo* studies are conducted to monitor the capacity of binders and also check their effect in the body and on other nutrients in food.

1.1.4 Silica and Alumina

Silica also known as silicon oxide (SiO2) is the name given to a group of minerals composed of silicon and oxygen, the two most abundant elements in the earth's crust. It can be added to food in very small quantity as an anticaking and conditioning agent. It can also act as an adsorbent and filter aid (Lewis and Harrison, 2010). An *in-vitro* study of the aflatoxin adsorption capacity of silica on naturally contaminated maize showed that silica can adsorb a ratio 63.6 to 84 ±1.2 % of AfB1 (Asghar *et al.,* 2015). However, the adsorption level is directly proportional to the amount of silica used for the experiment. Alumina (Al_2O_3) or Aluminum Oxide is the only oxide formed by the metal aluminum and occurs in nature as the minerals corundum (Al_2O_3); diaspore (Al_2O_3•H_2O); gibbsite (Al_2O_3•$3H_2O$); and most commonly as bauxite, which is an impure form of gibbsite. Bauxite is named as one of the natural resources Ghana is blessed with. According to

Miyazakai and Balint (2011), Alumina is one of the most widely used adsorbent for removal of dissolved pollutants from waste water. Among other toxic wastes, alumina can be used to remove PCBs, lead, arsenic, flouride etc.

Since most of the inorganic binders are alumno-silicates, their main components are alumina and silica. For instance, the chemical composition of montmorillonite contains 54.6 - 65.6 % SiO_2, 14.5 - 19.7 % Al_2O_3 (Marroquin-Cardona *et al.,* 2009). *In-vitro* studies and subsequent *in - vivo* studies are used to screen adsorbents to ascertain their ability to adsorb aflatoxins. The *in-vitro* studies usually come first preferably they provide a cheaper and less demanding base or standing ground for in depth study on the adsorbents. The adsorption capacity of different adsorbents was studied by Huwig *et al.* (2001). According to the study, activated charcoal adsorbed 10 mg/g of aflatoxin, alumino-silicates <0.02 mg/g, HSCAS 86 mg/g, montmorillonite 1.9 mg/g, phyllosilicates and bentonites 0.03 – 0.44 mg/g, diatomaceous earth 0.5 – 1.5 mg/, other alumino-silicates such as ethical, novasil, perlite and zeolite could adsorb aflatoxin in the range of 0.06 – 0.80 µg/g.

1.2 PROBLEM STATEMENT AND JUSTIFICATION

A review by Williams *et al.* (2004) revealed that about 4.5 billion persons in developing countries are chronically exposed to largely uncontrolled amounts of aflatoxin. The study also claimed that exposure to and toxic effects of aflatoxin on immunity and nutrition in these countries account for the short life span of the people and 40% of their disease burden. This however can easily be explained because of subsistence farming, small scale food industries and food insecurity prevalent in these countries. A lot of people will rather just eat than think of aflatoxin limits in their foods. The issue of throwing away contaminated foods does not really come up hence the need for more practicable means of preventing biological exposure of aflatoxin in foods.

Several adsorbents, mostly clayey materials such as zeolites, bentonites and other aluminosilicates have been studied *in-vitro* and *in-vivo* and were found to effectively adsorb aflatoxin and protect animals from its dire effects of aflatoxicosis. However, availability and cost of the binders is a necessary factor. For example, if the adsorbent has to be imported before it can be used or if the process of its synthesis is complicated, then the purpose is defeated already. It therefore becomes relevant to also study some inorganic materials abundant in our environment such as alumina which can be synthesized from bauxite and silica obtained from quartz to ascertain if they have any potential in aflatoxin adsorption.

The use of inorganic adsorptive compounds to reduce the adsorption of aflatoxin from the gastrointestinal tract is cheap and applicable hence its popularity and attention from researchers. However, it is important that the availability of the binder is established so that it is handy when needed and does not have to be imported. Also, a composite binder or modified ones are likely to bind aflatoxin better than the single ones. This work therefore concentrates on studying the potential of a composite binder made from alumina and silica as a contribution to the needed intervention for aflatoxins. This work is beneficial because it will create awareness about some inorganic materials in the environment that can possibly adsorb mycotoxins. It would open a door for the various possibilities of alumina and silica in binding not only aflatoxins but other hazards and impurities in food and feed and will also add more knowledge to the existing ones on the effect of heat in improving binding capacity of inorganic binders.

1.3 OBJECTIVE

The main objective of this project was to synthesize alumina-silica complexes and predict a model for optimum conditions for *in-vitro* aflatoxin adsorption by the complexes.

CHAPTER TWO

LITERATURE REVIEW

2.1 AFLATOXICOSIS

Aflatoxin is a recognized risk factor for cancer and other toxic effects. It can cause certain adverse immunological and nutritional effects depending on the level of exposure (Williams *et al*., 2004). There are many types of aflatoxins but the primary ones include B1, B2, G1, and G2 (Hussein and Brasel, 2001). Aflatoxin B1 (AfB1) and mixtures of aflatoxin G1, B1, and M1 are proven human carcinogens (IARC, 1993). The most abundant in nature and toxic of all the aflatoxins known is the AfB1. **Figure 2-1** shows the structure of the different types of aflatoxins. Aspergillus can be detected on commodities such as corn using fluorescence which differentiates between B1 and B2 (blue) or G1 and G2 (green). Biomarkers of exposure and effect of AF in humans may include presence of AFB1 in the urine, DNA mutation, AfB1-DNA adduct formation and decrease in salivary IgA levels (IARC, 1993). Physical evidence of the presence of fungus may be visible to the naked eyes, yet there are often some toxic levels of aflatoxin in foods which may escape visual detection. This indicates that they pose significant health risks for humans.

2.1.1 Metabolism of Aflatoxin

In order for aflatoxin to exert its toxic effects, it must be bioactivated in the system. Researchers have focused on the biotransformation pathways for AFB1 since it is the most prevalent of all the aflatoxins (Romoser *et al.,* 2013). The metabolism of AFB1 is activated by cytochrome P450 enzymes forming the AFB1-8,9-epoxide, which is capable of covalent modification of DNA, preferentially at the N7 position of guanine. The resulting adduct is however able to undergo a base-catalyzed reaction during which it opens up the imidazole ring of dG to form stable 8,9dihydro-8(2,6-diamino-4-oxo-3,4-dihydropyrimid-5-yl-formamido)-9-hydroxyaflatoxinB1 (AFB1-FAPY) adduct. The AFB1-FAPY adduct has been found by studies to be six times more

mutagenic than the others. Therefore, it has the ability to cause a much stronger blocking effect on DNA replication than the AFB1-N7-dG adducts (Alekseyev *et al*., 2004). One of the difficulties in handling aflatoxicosis is that aflatoxins are very stable and most likely to remain intact even at a temperature as high as 250 ºC. Foods susceptible to Aflatoxin include maize, peanuts, cottonseed, millet, sorghum, groundnuts, chili, yam products (chips, flour), tiger-nuts and other feed grains. An estimate by the Food and Agriculture Organization (FAO) shows that the worldwide losses of foodstuff which is about 1000 million metric tons every year has the contamination of basic foods with mycotoxin producing fungi as a contributing factor (Bhat *et al*., 2010).

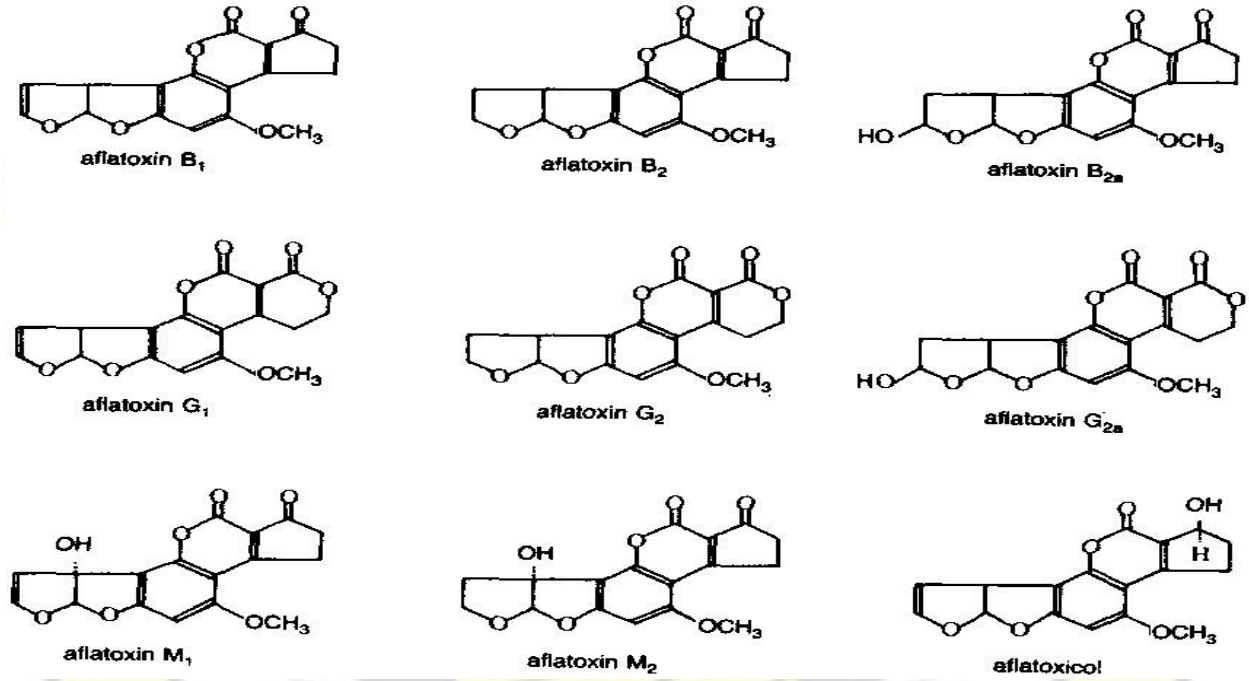

Figure 2-1: Molecular structures of aflatoxins (Goto and Marble ,1989).

A study by Jolly *et al*. (2006) showed that aflatoxin B1 albumin adduct levels in the population of Kumasi, Ghana is significantly associated to socio-demographic factors, such as educational level, ethnic group, geographic location household size, and educational level of children in the household. The study suggested that the higher the educational level of the populace, the more

they are exposed to knowledge about food contamination and are therefore more equipped to make informed choices about the food they eat. However, it is a fact that the non-educated populaces are still more in number as far as developing or under-developed countries are involved. Suggesting that the number of people in danger of exposure to aflatoxins are more than those said to be safe. It is important to continue the awareness on the danger of aflatoxins as researchers continue to study inexpensive but effective ways of eliminating this danger of aflatoxicoses.

2.2 AFLATOXIN EXPOSURE IN DEVELOPING COUNTRIES

The scarcity of food and unstable state of the economy has made the approaches of developed countries to the management of aflatoxins unachievable in developing-country settings. While developed countries have working economic systems and agricultural infrastructures in place to reduce aflatoxin exposure to the barest minimum, developing countries can barely find enough food for the populace let alone having infrastructures. A review of Aflatoxin contamination in developing countries showed that over four billion persons living in developing countries are chronically exposed to largely uncontrolled amounts of the toxin (Williams *et al*., 2004). In these countries, climate and the crop storage conditions encourage fungal growth and hence the occurrence of mycotoxins. Majority of the populace depend on subsistence farming or unregulated local market (Shephard, 2008). The incidence of mycotoxins in developing countries has also been reviewed showing aflatoxins as having the highest incidence amongst others (**figure 2-2**). Exposure to mycotoxins places a significant restriction on the effort to improve human health in developing countries (Shephard, 2008). Maize and maize products were tested in Ghana and results showed that all the maize samples collected from silos and warehouses contained aflatoxins at levels ranging from 20–355 µg/kg, fermented maize dough collected from major processing sites

contained aflatoxin levels of 0.7–313 µg/kg (Kpodo, 1996). Death cases and exposure of the population in Kenya to aflatoxicosis led to a survey by Lewis *et al.* (1995). Findings demonstrated widespread aflatoxin contamination of maize within the regional market distribution system. An alarming proportion (55 %) of maize samples from markets in all four study districts had aflatoxin levels greater than the regulatory standard of 20 ppb.

A cross-sectional study on 480 children aged from 9 months to 5 years from Benin and Togo showed aflatoxin-albumin adducts detection in 475/479 (which equals to 99 %) samples. It was also noted in the study that the children mostly affected where the ones that have been weaned and that were introduced to solid food since the toxin level in breast milk is much lower than that in weaning and adult foods. The problem of stunted growth and under-weight was also related to aflatoxin exposure (Gong *et al.*, 2002).

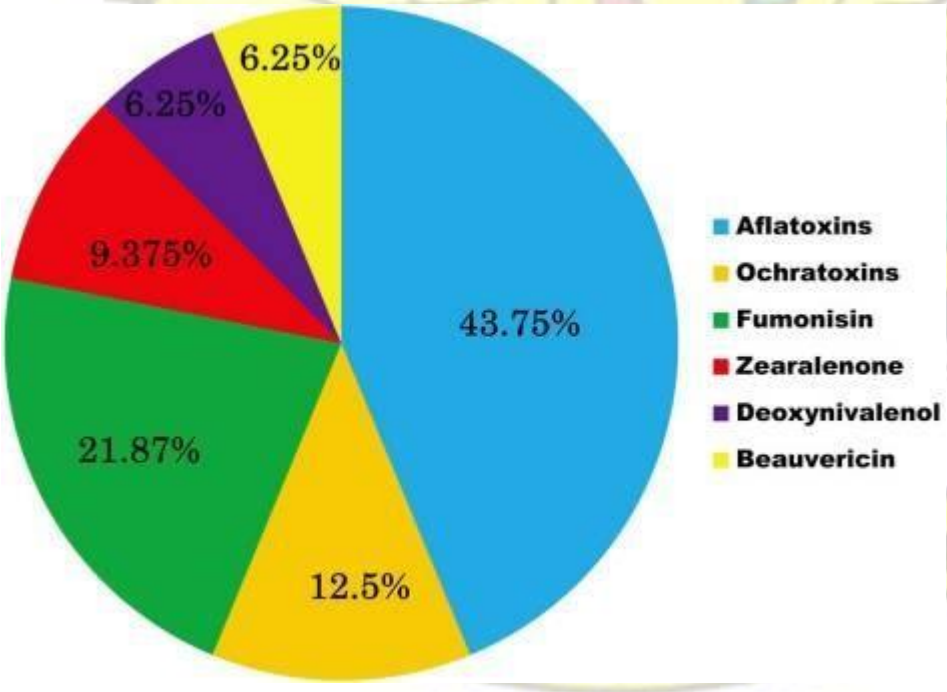

Figure 2-2: Distribution of mycotoxins in African countries (Darwish *et al*., 2014)

Since many people in developing countries are chronically exposed to aflatoxin contamination, the prevention and control study of the toxin cannot be over-emphasized.

2.3 INTERVENTION FOR AFLATOXIN

The prevention and control of aflatoxin has now become very important in order to promote quality of the food we eat. For developing countries, this would mean more than protecting consumer health. It would also promote international trade as farmers would no longer worry about having their goods rejected at the ports due to aflatoxin contamination. Otsuki *et al*. (2001) made an estimation of $670 million as losses that African countries incur due to the inability of their farmers to meet aflatoxin standards for the crops they produce. As the awareness of the health implications of aflatoxins increase, so does the effort made to prevent its occurrence, reduce its content in foods and feeds and also prevent its bioavailability in the body when ingested. Physical, chemical and biological interventive procedures have been studied even though the most effective intervention is definitely preventing its occurrence in foods and feeds (Rustom, 1997). From literature, it is seen that there are three ways by which aflatoxins can be combated. These involve: 1.) Preventing contamination while the crops are on the field or while being stored. 2.) Decontaminating already contaminated crops. 3.) Adsorbing the aflatoxin that has been ingested already making its toxic effects unavailable to the body.

Some researchers in Kenya (Lewis *et al.*, 1995) found that to effectively prevent future outbreaks of aflatoxicosis, establishment of long term interventions such as a comprehensive food safety program must be implemented. These interventions must target both market vendors and local

farmers in order to prevent or minimize future outbreaks of aflatoxicosis and reduce long-term exposure to aflatoxins.

Technologies have been developed which helps to prevent, control or counteract aflatoxin contamination of crops in the field or in storage. There is physical treatment, chemical treatment, biological control and the inclusion of organic and inorganic binders. This may include the introduction of non-toxic strains of aflatoxin which will compete with the toxic strains and also the use of aflatoxin-resistant varieties of crops especially maize though these varieties have low commercial values (Bandyopadhyay and Cotty, 2013). The feasibility of any aflatoxin intervention strategy may be technically measured using four components: 1) characteristics of the basic intervention, 2) characteristics of delivery, 3) requirements on government capacity and 4) usage characteristics (Wu and Khlangwiset, 2010).

2.3.1 Physical Control

Vincelli *et al.* (1995) reported that cleaning corn kernels and also sorting out the damaged ones help to reduce aflatoxin concentrations. However, some whole kernels may also contain high concentrations of aflatoxin. Postharvest aflatoxin accumulation remains a threat in developing countries. Magan and Aldred (2007) studied postharvest strategies in handling crops and found that the control points, which are key in effective produce handling include proper harvesting, drying of the crops to reduce moisture content which encourages fungal and mould growth, and storage stages should be well studied and practiced. Jouany (2007) suggests that washing, polishing, mechanical sorting and separation, density segregation, flotation, autoclaving, roasting and microwave heating, UV irradiation, ultrasound treatment and solvent extraction are also

effective in combating mycotoxins. However, these techniques will be efficient depending on the level of mycotoxin contamination of the food.

Essential oils such as oils from *coriandrum sativum*, *Melissa officinalis* L, *Thymus vulgaris*, *cinnamomum zeylanicum* e.t.c. due to their high anti-oxidant properties are able to reduce contamination by *fusarium* or *aspergillus* and as such can be recommended as natural preservatives during cereal storage. These oils in themselves have no toxic effects in the body and are biodegradable (Sumalan *et al.,* 2013).

2.3.2 Biological Control

Microorganisms can be used for mycotoxin degradation biologically. These microorganisms may include bacteria found in either soil or water, protozoa, fungi and some enzymes. The efficiency of this type of microbiological treatment may vary from less-toxic to non – toxic products (Aliabadi *et al*., 2013). The review also showed that microorganisms can be used on a large scale to reduce to the barest minimum the agricultural losses caused by aflatoxin contamination of crops and also protect the health of both animals and humans. The isolates of microorganisms such as *Stenotrophomonas maltophilia*, *Bacillus sp*., *Enterobacter sp*., *Rhodococcus sp.*, *Brevundimonas sp., Klebisiella sp., Cellulosimicrobium sp.* from different sources were able to degrade aflatoxin at percentages between 73.75 % and 82.50 %.

2.3.3 Chemical Control

Chemical control of aflatoxin involves using chemicals such as acids, bases, salts, oxidizing and reducing agents and other substances. However, just few of these chemicals have been able degrade aflatoxin without an equal adverse effect on the nutritional and organoleptic qualities of the food. Chemical methods are very demanding and costly because not only are expensive

chemicals needed, other treatments such as drying and cleaning are also required to complete the process. Although some chemicals such as monoethylamine, calcium hydroxide, ammonia has been used to destroy aflatoxins, ammoniation is the approved chemical method for annihilating aflatoxins in feeds. The set back of this method however is the likely health decline of the animals due to too much ammonia residues in their system (Kolossova *et al.,* 2009).

2.4 AFLATOXIN ADSORBENTS

According to Murugesan *et al*. (2015), the best known method for mycotoxin deactivation is with the use of adsorbents, which are known as mycotoxin binders, sequestering agents or enterosorbents. Adsorption can be described as a process by which dissolved material either in liquid or gaseous state is bound to the surface of another and may be expressed in terms of adsorptive surface area per unit mass. These binders may be of microbial (organic) or inorganic nature which includes charcoal and clay minerals. The addition of binding agents to foods and feeds has received a lot of attention in the past years. The binders are meant to act like a 'chemical sponge' which will practically 'mop up' the mycotoxins from the gastrointestinal tract thereby preventing it from being available to other target organs in the body. The chemical structure of both the mycotoxin and the binder determines the effectiveness of adsorption. The important characteristics of the adsorbent to be taken into consideration include its physical structure, its charge, pore size and surface area (Kolossova *et al.,* 2009).

2.4.1 Organic Binders

Microorganisms found in the soil or water such as bacteria, fungi, and protozoa and specific enzymes when isolated are capable of degrading aflatoxin. Some aflatoxin-producing fungi from *Aspergillus* species have the ability to degrade their own synthesized mycotoxins. Yeasts and lactic

acid bacteria have been investigated and found to work as biological binders that adsorb aflatoxins preventing their availability in the gastro - intestinal tract of both humans and animals (Aliabadi *et al.*, 2013).

Surface binding of aflatoxin B1 by lactic acid bacteria (LAB) has been demonstrated by repetitive aqueous extraction. The study showed that 71% of the total AFB1 remained bound by the fifth extraction. Mainly, the binding of the aflatoxin occurs extracellularly on the cell wall of the bacteria (Haskard *et al.*, 2001). Organic polymers have also been used as mycotoxin binders. These may include complex indigestible carbohydrates and synthetic polymers such as cholestryamine and polyvinylpyrrolidone can adsorb mycotoxins.

2.4.2 Inorganic Binders

NovaSil Clay (NS): This enterosorbent also known as calcium montmorillonite has received a lot of attention due to the promise it shows in the human trials for aflatoxin intervention. It is perceived that this clay will come in handy in situations of high aflatoxin contamination of foods available to people at a particular moment to reduce risk. (Wu and Khlangwiset, 2010). It has been proven to bind aflatoxin with high affinity and high capacity in the gastro intestinal tract of many animal species thereby preventing aflatoxicosis (Phillips *et al*., 2008). A study carried out in Ghana by Wang *et al*. (2008) within a space of three months revealed that capsules containing NovaSil clay can be used to effectively reduce aflatoxin bioavailability. The inclusion of NovaSil clay at a low level of 0.5 % in the aflatoxin contaminated diet of animals that are sensitive to the toxin is capable of reducing the health effects of aflatoxicosis (Phillips *et al.*, 1995).

Activated Carbon*:* Activated charcoal is simply burnt wood (or other materials) excluding oxygen through controlled oxidation and or processing by steam. Common materials from which activated charcoal may be processed include hard wood trees or coconut shells. Activated charcoal is

considered to be medicine's most powerful adsorbent and as such, it readily works to adsorb many toxins, poisons and heavy metals from the body, rendering them harmless. It has this amazing ability to bind or attract substances to itself while it exits the body. Kana *et al.* (2010; 2011) reported that charcoals synthesized from Canarium seed and maize cob were able to promote growth performances of broiler chickens fed on aflatoxin B1-contaminated feed. Effectiveness of yeast, zeolite and active charcoal as aflatoxin absorbents in broiler diets have been evaluated by Khadem *et al.* (2012) and results showed that the mixtures of the absorbents were more effective in binding aflatoxin B1. Decker (1980) suggested that activated carbon might be used to prevent animal and human absorption of aflatoxins from contaminated foodstuffs since the addition of 100 mg of activated carbon (Norit-A) from aqueous media at pH 7 could adsorb 1 mg AfB1.

Zeolite: Zeolites are microporous minerals with a well-defined crystalline structure. They belong to a group of natural alumino-silicates. Generally, they contain silicon, aluminum and oxygen in their framework and cations, water and/or other molecules within their pores. There are three (3) main properties of zeolites which are related to both its structure and chemistry. These properties include adsorption, ion exchange and catalytic activity. The adsorptive property of zeolite has brought about its ability to adsorb mycotoxins. A high affinity addition of hydrated sodium calcium alumino-silicate (HSCAS), a compound obtained from natural zeolite to feedstuffs contaminated with aflatoxin has been shown to have a protective effect against the development of aflatoxicosis in farm animals as reported by Ramos and Hernandez (1997).

Bentonite: Bentonite is a highly colloidal rock which contains mainly montmorillonite, and may also contain feldspar, cristobalite and crystalline quartz. It has a very high cation exchange capacity amongst its other desirable properties (WHO, 2005). Bentonite is a mineral adsorbent which has been found with a high capacity to adsorb aflatoxins. It has a crystal structure which consists

mainly of alumina and silica tetrahedrons. It is used in animal feeds mostly to strengthen pellets and adsorb mycotoxins. According to Huwig *et al.* (2001), its adsorption capacity can be rated between 90 to 95 percent. *In-vitro* systems demonstrated the ability of bentonite to adsorb aflatoxins in aqueous media at different pH values and to a lower degree in gastric juice. While *in-vivo* data showed a significant reduction in AfB1 via milk excretion by 0.03–1 % bentonite in dairy cows (< 5 µg AfB1/kg feed). Since *in-vitro* systems do not completely mimic the complex situations during digestion, an efficacy assessment requires a minimum of two *in - vivo* studies to further validate the binder.

Hydrated Sodium Calcium Aluminosilicate (HSCAS): This binder is seen as the most effective adsorbent used for aflatoxin adsorption. As most other adsorbent, HSCAS was already sold as an anti-caking agent for animal feeds. It has been well studied and found to be able to form a stable complex with aflatoxin at temperatures between 25 ºC and 37 ºC. The addition of HSCAS at a percentage between 0.5 – 2 % in feed is able to adsorb up to 7.5 mg/kg of AFB1 in the feed. However, it has great affinity for aflatoxin and may not be effective on other mycotoxins in the food (Phillips *et al*., 1988).

Montmorillonite: Montmorillonite is a clayey substance that is formed by layers of aluminum and silica at a ratio of 1:2. Ramos and Hernandez (1996) studied *in-vitro* adsorption of aflatoxin using montmorillonite silicate. Affinity and capacity of the binder on four major naturally occurring aflatoxins are 1000 µg Aflatoxin B1, 425–450 µg Aflatoxin G1, 230 µg Aflatoxin G2 and 200 µg aflatoxin B2 per one gram of montmorillonite.

Kaolin: Kaolin which is also referred to as china clay is a mixture of different minerals. Apart from its main component being kaolinite, it may also contain mica, feldspar, illite, quarts, and montmorillonite. The structure of kaolinite which is the main component of kaolin is a tetrahedral silica sheet alternating with an octahedral alumina sheet. Kaolin may also contain substantial

amounts of quartz up to 58 % as its major constituent (Rees *et al*., 1992). Both human beings and animals have been observed to deliberately consume unpalatable soils especially clays with high content of kaolin. This behavior is known as *geophagy* (Trckova *et al*., 2004). A study by Hesham *et al*. (2004) showed that the addition of kaolin and activated charcoal to the aflatoxin contaminated diet at 0.5% level is capable of reducing mortality rate of the broilers, improve their body weight gain and increase the efficiency of feed utilization. Aflatoxin residues were not found in the birds after treatment with the binders.

2.5 IMPORTANCE OF INVITRO STUDIES ON AFLATOXIN ADSORPTION

An *in-vitro* study refers to the studies of biological properties that are done in a test tube rather than in human or an animal. The importance of i*n-vitro* study in mycotoxin adsorption cannot be over–emphasized because an adsorbent has little or no ability to do well *in-vivo* if it does not adsorb mycotoxin *in-vitro*. The tests are less expensive and can be done with fewer ethical issues unlike *in-vivo* study which is very expensive (Boskey, 2016). *In-vitro* laboratory techniques are used to identify adsorbents that deserve further studies and also to predict the conditions best favorable for adsorption (Diaz and Smith, 2005). The necessity of *in-vitro* studies prior to the *invivo* tests of adsorbents was echoed by the study of Vekiru *et al.* (2014) where the binders that were seen to do very well in adsorbing aflatoxins *in-vitro* also did well *in-vivo*. However, there are some exceptions in literature to this statement. It therefore becomes necessary to carry out series of tests to validate results of adsorption. There are four different types of *in-vitro* studies which includes 1) Single – concentration studies 2) Adsorption isotherms 3) Adsorption isotherms in the presence of a food matrix 4) Static and dynamic gastro – intestinal experimental models.

Table 2-1: Results of in-vitro single concentration studies on aflatoxin adsorption.

	Binder	Binder Concentration (g)	Toxin Concentration (µg)	pH	Temperature (°C)	Binding (%)
AfB1	Clinoptilolite	0.2	7.86	-	-	5.6
AfB1	HSCAS	0.2	7.86	-	-	91.3
AfB1	Charcoal	0.2	7.86	-	-	92.9
AfB1	Montmorillonite nanocomposite	5	100	7	37	90
AfB2	Montmorillonite nanocomposite	5	100	7	37	83
AfG1	Montmorillonite nanocomposite	5	100	7	37	82
AfG2	Montmorillonite nanocomposite	5	100	7	37	73

Source: EFSA (2009).

2.5.1 Single Concentration

The single concentration method was used for the purpose of this study. It is the simplest method of all the types of *in-vitro* studies and therefore most widely used. It is popular because the amount of toxins used is not much thereby reducing wastes. A known amount of the toxin is usually reacted with some portion of the adsorbent in an aqueous solution. Results from this type of test are usually reported as percentage adsorption (EFSA, 2009).

2.5.2 Adsorption Isotherms

Isotherm studies make good use of the fact that adsorption is a reversible process. A plot of the amount of mycotoxin adsorbed per unit weight against the concentration of the mycotoxin is utilized. The isotherm curves derived are used to determine capacity of sequestering agent and their affinity for the mycotoxin only.

A chemosorption index which is defined as amount of toxin left adsorbed after extraction divided by the total amount of initially present is used to account for adsorbent stability (EFSA, 2009).

Result of some single concentration *in-vitro* studies carried out by researchers (Lemke *et al*., 2001 and Shi *et al.*, 2006) are displayed in the table below. The solvent used for these experiments was water.

2.6 HEAT TREATMENT OF ADSORBENTS

The effect of heat treatment of binders on the adsorption of mycotoxins varies according to the type and property of the adsorbent. Study by Wongtangtintan *et al*. (2014) showed that Thai bentonite clay from Lopburi province in Thailand is capable of adsorbing AfB1 with the highest adsorption capacity at 600 °C. The study also revealed that when the clay was heated above 700

°C, the adsorption capacity started declining. This fact about heated bentonite was reiterated by the study of Nones *et al.* (2015) where the adsorptive capacity of the clay progressively reduced with increase in temperature. These differences observed with different temperature shows that heat affects the interlayer spaces of the clay and subsequently its adsorptive capacity.

2.7 RESPONSE SURFACE METHODOLOGY

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for the developing, improving and optimizing processes. It can also be used when developing or formulating new products or improving already existing product design (Myers *et al.*, 2009). Optimization of a product is an effective strategy of accomplishing its successful development (Granato and Calado, 2014). The development of food products and processes, if not systematically approached can be complex, expensive and may result to a total waste of time and resources in some cases. RSM is therefore very useful in decreasing the volume of experiments, reagents, time, financial input, energy, among others (Montgomery, 2009). Some researchers, such as Amponsah *et al*. (2010) and Kurek *et al*. (2016) have utilized the RSM to study food products and processes. The independent variables are usually set at a range and the design is used to predict optimum conditions for the desired response. The response is in effect modelled by factorial techniques and ANOVA, but these are extended for more detailed modelling of the effect (Bower, 2013).

A study was conducted by Ansari *et al*. (2015) on modelling of aflatoxin G1 (AfG1) reduction by kefir grain using response surface methodology. In the study, the central composite design was applied and the model provided a good prediction of aflatoxin G1reduction under the assay conditions. Independent variables were toxin concentration, kefir-grain level, contact time and

incubation temperature. Results from the optimization showed that optimum conditions of the variables will give 96.8% AfG1 reduction.

2.8 FURTHER OUTLOOK

The intervention process for the occurrence of aflatoxin has over the years been concentrated on prevention and control. For developing countries, it is important to have a ready and applicable plan for when aflatoxins does occur in foods in order to avoid risk of aflatoxicosis or food wastage. The use of binders has been named by researchers as the best control strategy for aflatoxicosis in animals as it prevents the toxin from being available to the human system after consumption. However, even though many clays and biological agents have been proven to bind aflatoxins, they will remain nice on paper only if acquiring it is difficult, mode of application is complex and/or it is expensive to purchase. As stated by Phillips *et al*. (2008), not all geographical regions in Africa have access to the type of clays that effectively bind aflatoxins. This means that in most cases, these binders need to be imported and therefore may not be available when needed. Therefore, it is important to continue to examine some available and cheap adsorbents in our environment for their possible aflatoxin adsorption capacities.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Alumina was purchased from BDH chemicals and silica chippings were obtained from the river bed at *Oda* river in *Apromase,* 12 km from KNUST. The silica obtained was ground and sieved to a size of ≤0.5 mm and kept in plastic bags pending further analysis. The micrographs of sample ASC was taken with Leica optical microscope (1250X, China and the length of the grains was determined.

3.2 METHODS

3.2.1 Design of Experiment

The experimental design for compositing the alumina-silica mixture was obtained using the Doptimal quadratic x quadratic, mixture-process design (Design Expert, 2008) where a total of 28 runs were generated. The mixture components which are alumina and silica were represented with letters A and B respectively. These components were varied at 20-60 % for alumina and 4080 % for silica. Table 3-1 indicates the summary of the specified levels of the mixture components and that of the process factors.

Table 3-1: Summary of the Mixture Components and the process factors

Component	Name	Units	Type	Minimum	Maximum
A	Alumina	%	Mixture	20	60
B	Silica	%	Mixture	40	80
C	Time	H	Numeric	0	4

D	Temperature	^{o}C	Numeric	0	1400

Two factors (temperature and time) represented with letters C and D respectively were also varied and used in the experimental design to generate values for the experimental runs.

Temperature in °C was varied ranging from 0 °C to 1400 °C and time was also varied (0 - 4 h). The different proportions of alumina and silica were measured (50 g) into crucibles and fired in Bionics Muffle furnace (BST/MF/1800, India) according to the conditions set for each of the 28 runs. Samples that have clumped together after been calcined at high temperatures were ground to powder of size 0.05 mm.

3.2.2 Screening of Alumina-Silicates Samples against Aflatoxin Bindability

Stock Extract Preparation: The stock extract from naturally aflatoxin contaminated peanut sample was prepared by weighing 25 g of the peanuts and blending this with 100 ml of 70 % methanol in a warring blender at high speed for 2 min. It was then filtered using fluted paper. Filtrate was then collected in a falcon tube. 100 µl of the filtrate was injected into the HPLC and analyzed to determine the total aflatoxin.

The binding process*:* The adsorption capacity of ASC on naturally contaminated peanut samples was evaluated by weighing 50 mg each of all the ASC samples into a centrifuge tube. Then, 3 ml of distilled water was added to the pre-weighed binders. The stock extract (2 ml) was pipetted into the tubes as well. The mixture was then vortexed for 1 min to homogenize and was further placed carefully in a rotator shaker at 300 rpm for 15 min. It was then immediately placed in the centrifuge at 3000 rpm for 5 min. Then, 100 µl of the clear supernatant was analyzed using the HPLC for residual (unbound) aflatoxin. This analysis was carried out in batches to ensure that approximately

equal contact time was apportioned for the binder and the stock solution. Negative control was prepared by taking the stock extract through the same *in-vitro* protocol but this time, without the binder.

The HPLC Determination*:* A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10 AxL fluorescence detector (Ex: 360 nm, Em: 435) with Phenomenex HyperClone BDS C18 Column (150 x 4.60 mm, 5 um) was used. The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1 ml/min with column temperature maintained at 40 °C. To 1 L of mobile phase were added 119 mg of potassium bromide and 350 µl of 4 M nitric acid (required for post column electrochemical derivatization with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G1, G2, B1, B2) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6 ng/µl in methanol. Concentration of B1 and G1 were 0.5, 1, 2, 8, 16 ng/g per 100 µl injection.

Concentration of B2 and G2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100 µl injection. Limit of Detection and Limit of Quantification were established at 0.5 ng/g and 1 ng/g respectively (AOAC, 2005). Percentage reduction of aflatoxin after the HPLC determination was calculated using the formula below:

$$\% \text{ Reduction} = [(Y - X) / Y] \times 100$$

Y and X represent the aflatoxin value of stock solution and the aflatoxin value of the stock solution after been treated with ASC respectively.

3.3 STATISTICAL ANALYSIS

Data from the aflatoxin determination were computed and the results plotted using DesignExpert (2008). The variations in the data collected such as coefficients of regression- (R^2), adjusted regression- (adj R^2), regression – (pred R^2), and adequate precision – (adeq precision) were studied.

After ANOVA studies of the model, the level of significance ($p<0.05$) were assessed as well as any interactions that occurred among the factors that were varied. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were plotted and performance of the factors and response made.

3.3.1 **Optimization of Process**

Numerical optimization of the process was carried out in order to determine the best treatment conditions that will give desirable response. The response factor which is the reduction of aflatoxin was to set maximum with the highest importance. The composition of the aluminosilicates and the treatment factors (temperature and time of heating) were all set in range as shown in **table 3-2**.

Table 3-2: Constraint table showing the importance level of factors and response and the goals set for optimizing conditions for aflatoxin adsorption

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Alumina	is in range	20	60	1	1	3
Silica	is in range	40	80	1	1	3
Time	is in range	0	4	1	1	3
Temperature	is in range	0	1400	1	1	3
% Reduction of TAfT	Maximize	33.74	77.83	10	1	5

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 MODEL FITTING

Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. It is used mainly where several inputs have the potential to affect performance of a product or a process (Myers *et al.,* 2009). In analysing an experiment, design expert fits models relating a response or quality characteristic to a set of controllable variables. The ability of alumina-silica complexes to bind aflatoxins is shown in **table 4-1** below. The R^2 value was 0.968014 which was close to the adjusted R^2 value of 0.907597. This value of R^2 shows a good fit of model since its very close to 1 which indicates unity. The Model F-value of 16.02 implies the model is significant. There is only a 0.01 % chance that the model F-value this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate that model terms are significant. The response function for the experiment was predicted by the following polynomial regression equation:

$$Y = \sum_{i=1}^{2} \beta_i x_i + \sum_{i=3}^{5} \beta_i x_1 x_{i-1} + \sum_{i=6}^{7} \beta_i x_2 x_{i-3} + \sum_{i=8}^{9} \beta_i x_1 x_2 x_{i-5} + \sum_{i=10}^{11} \beta_i x_3 x_4 x_{i-9} + \sum_{i=12}^{13} \beta_i x_1 x^2{}_{i-9} + \sum_{i=14}^{15} \beta_i x_2 x^2{}_{i-11} + \sum_{i=16}^{16} \beta_i x_{i-15} x_{i-14} x_{i-13} x_{i-12} + \sum_{i=17}^{18} \beta_i x_1 x_2 x^2{}_{i-14}$$

Y= Dependent variable (% Reduction of TAfT), x_1= composition of alumina, x_2= composition of silica, x_3= time, x_4= temperature and β_i = coefficient of the independent variables.

Table 4-1: Experimental runs and percentage reduction in total aflatoxin

Run	Component 1 A:Alumina %	Component 2 B:Silica %	Factor 3 C:Time H	Factor 4 D:Temperature ◦C	Actual Response Reduction of TAfT %	Predicted Response Reduction of TAfT %
1	20	80	0	800	55.67	50.62
2	40	60	4	1400	45.45	45.24
3	60	40	4	1400	54.96	56.00
4	30	70	1	400	76.45	79.66
5	30	70	4	800	51.90	53.85
6	40	60	0	0	77.83	77.66
7	40	60	0	1400	53.10	54.28
8	50	50	2	1200	76.47	74.06
9	20	80	2	1400	51.39	47.00
10	60	40	1	800	33.74	39.62
11	20	80	2	800	35.54	38.72
12	20	80	4	1400	77.39	78.23
13	60	40	4	0	76.25	79.76
14	20	80	0	0	76.29	76.80
15	60	40	0	1400	50.22	49.32
16	60	40	4	1400	54.96	56.00
17	20	80	4	0	76.29	75.58
18	40	60	0	800	55.17	53.25
19	20	80	4	0	76.29	75.58
20	60	40	2	0	76.25	73.04
21	20	80	0	1400	48.47	52.05
22	60	40	0	0	76.25	76.12
23	60	40	4	800	51.81	47.15
24	40	60	4	0	77.38	76.42
25	60	40	0	1400	50.22	49.32

26	20	80	0	0	76.29	76.80
27	60	40	0	0	76.25	76.12
28	40	60	2	0	*77.38	50.62

*Outlier

4.3.1 ADSORPTION CAPACITY OF ASC

The results indicated that ASC has the capacity to adsorb aflatoxin *in-vitro*. Aflatoxin value for the stock solution of naturally contaminated peanut sample was 456.27 ppb. There was noticeable decrease in aflatoxin level of stock solution containing naturally contaminated peanuts. However, there is no significant difference between ASC heated and non-heated in the adsorption of aflatoxin as the highest noticed reduction in aflatoxin (77.83 %) was found with a combination of 40 % alumina and 60 % silica with no heat treatment. While the lowest adsorption performance of ASC (33.74 %) was found with 60 % Alumina and 40 % silica heated at a temperature of 800 °C for 1 h. Some combinations of ASC were found to adsorb aflatoxin and give reductions of the toxin up to 77.39 % at a temperature of 1400 °C.

Interaction time between ASC and aflatoxin extract was approximately 25 min. Clayey binders are likely to reach stability and attain full adsorptive potential if the interaction time is prolonged. A study by Asghar *et al*. (2015) showed that interaction time is directly proportional to silica adsorption of aflatoxin implying that as the interaction time increased, so did the adsorption capacity.

4.4 ANALYSIS OF VARIANCE

The results from the ANOVA table show that the quadratic model used for the analysis was significant. The values under the p-value less than 0.05 confidence level are significant while those above the value are non-significant. From the ANOVA table, A stands for alumina, B for Silica, C for time and D for temperature. Values greater than 0.10 indicate the model terms are not significant. Model reduction is used to improve models when there are many insignificant terms. The linear interaction between alumina and silica has a p-value of 0.0001 indicating that the combination ratios of alumina and silica is significant. This means that more of alumina and

less of silica or vice-versa has an effect on the *in-vitro* analysis. The linear interaction between alumina and time of heating (p-value of 0.15) shows that these components have no significant effect on the model. The interaction between heating time and concentration of silica was significant but on the contrary, the interaction between silica and heating temperature was not significant. Indicating that heat treatment of silica does not significantly affect its adsorptive capacity. This is not the case for alumina as the results show that temperature has a significant effect on its performance. Time of heating does not affect the performance of ASC significantly. The temperature changes have no significant effect on ASC according to the ANOVA table. The regression table suggests that combinations of heating time and temperature have no significant effect on adsorption capacity of alumina. The interaction between silica, temperature and heating time shows significance in the model. However, the interaction between all the components shows significant relationship thereby making the model relevant. It can be inferred from the ANOVA results that the heating time and temperature has a significant effect on the adsorptive capacity of ASC. However, the heating temperature significantly affects the silica component while its effect on alumina is not significant.

Table 4-2: ANOVA for response surface quadratic model for aflatoxin reduction response for the treatment conditions of ASC

Source	Sum of squares	Df	Mean square	F value	p-value	
Model	5405.14	17	317.95	16.33	0.0001	Significant
1linear mixture	45.33	1	45.33	2.33	0.1614	
AB	1186.32	1	1186.32	60.92	0.0001	
AC	49.01	1	49.01	2.52	0.1471	
AD	1102.12	1	1102.12	56.59	0.0001	
BC	221.18	1	221.18	11.36	0.0083	
BD	179.79	1	179.79	9.23	0.0141	
ABC	175.55	1	175.55	9.01	0.0149	
ABD	68.85	1	68.85	3.54	0.0928	
ACD	5.42	1	5.42	0.28	0.6106	
BCD	243.89	1	243.89	12.52	0.0063	
AC^2	26.42	1	26.42	1.36	0.274	
AD^2	508.31	1	508.31	26.1	0.0006	
BC^2	394.04	1	394.04	20.23	0.0015	
BD^2	197.27	1	197.27	10.13	0.0111	
ABCD	101.44	1	101.44	5.21	0.0484	
ABC^2	1028.38	1	1028.38	52.81	0.0001	
ABD^2	12.77	1	12.77	0.66	0.439	
Residual	175.27	9	19.47			
Lack of Fit	175.27	4	43.82			
Pure Error	0	5	0			
Cor Total	5580.41	26				

4.4 OPTIMAL CONDITIONS FOR ASC

One of the main objectives of RSM is the determination of the optimum settings of the control variables that result in a maximum (or a minimum) response. An adequate representation of the optimization conditions depends on the 'good' fitting of the model used (Khuri and Mukhopadhyay, 2010). About 100 numerical solutions were generated for optimal conditions. The large number of possible optimum conditions shows that alumina, silica and ASC emphasizes the effectiveness of alumina, silica and their complexes in binding aflatoxin. The selected optimal conditions given in **table 4-3** below shows promise both economically and realistically.

Table 4-3: Selected optimal conditions for ASC maximum adsorption of aflatoxin

Alumina (%)	Silica (%)	Time (h)	Temperature (°C)	% Reduction of TAfT
31.78	68.22	3.23	347.41	78.80

Silica is cheap, abundant and can easily be acquired along river beds. Therefore, it is only logical that the chosen combination of alumina and silica should contain more of the latter. The suggested heating temperature is below 350 °C and is cheaper compared to the higher temperatures (800-1400 °C). Although the ASC can also effectively bind aflatoxins even when not heated, consideration is given to where silica is gotten from. Heating the binders at a low temperature will help to eliminate some impurities such as microorganisms before it is added to food.

4.5 MODEL GRAPHS

Design Expert offers a wide range of different plots to show how the response varies with changes in the controls. The contour and 3D surface plots are only appropriate for continuous control variables (Buxton, 2007). The model graphs analyze results by keeping one of the factors constant while showing its interaction with other factors towards the response which in this case is the reduction of TAfT. The above information for the optimum conditions (**table 4-3)** was used to generate contour and 3D graphs. The components considered were (alumina: silica, temperature and time) and one response (% reduction in aflatoxin). The contour and 3D graphs are laid side by side in order to clearly report the interactions. The red coloured areas on the model graphs represent the highest adsorption capacity, green represents medium adsorption and the blue colour represents the lowest.

4.5.1 Effect of ASC compositions on Aflatoxin Adsorption

The **figure (4-1)** below shows that at an alumina: silica ratio of 32:68, the adsorption capacity of ASC increases with decrease in temperature and also as heating time increases.

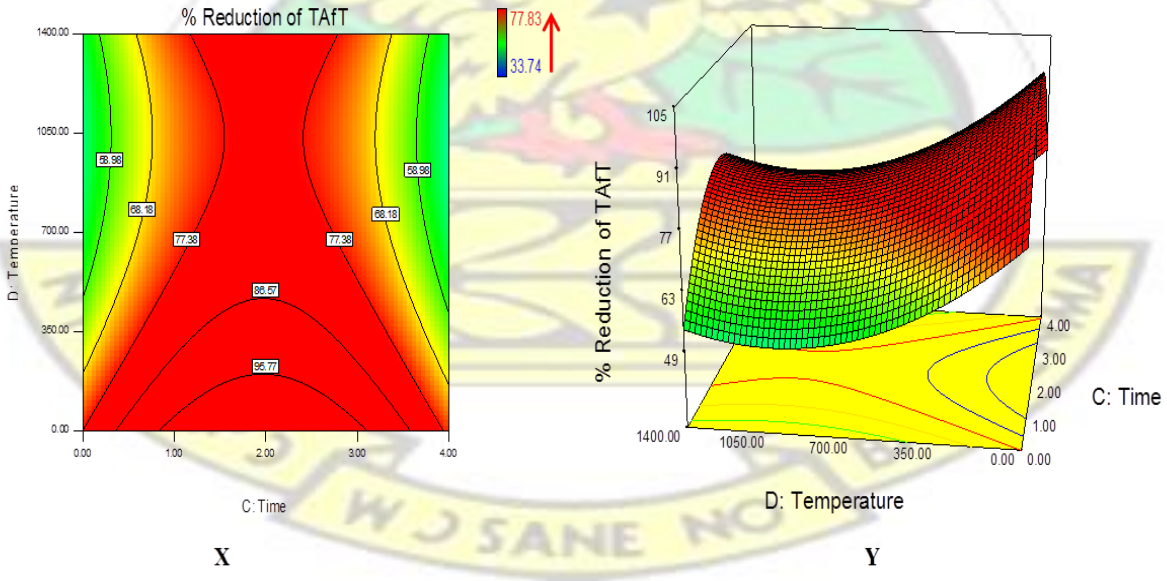

Fig 4-1: contour (X) and 3D (Y) graphs showing interactions between optimal conditions at a fixed alumina:silica ratio of 32:68.

From the contour graph (X), the red colour covers the base and commences a curve-in at a temperature of 700 °C. This curve-in closes more at temperature of about 1200 °C and spreads out a little more as it approaches 1400 °C. This behavior suggests that at 1400 °C, ASC adsorbs better than at temperatures ranging from 800 – 1200 °C. This may be due to grain development and growth to a relatively large size associated with alumina and silica heated at high temperatures ranging from 1400-1600 °C (Sacks *et al*., 1997).

4.5.2 Effect of Temperature on Aflatoxin Adsorption Capacity of ASC

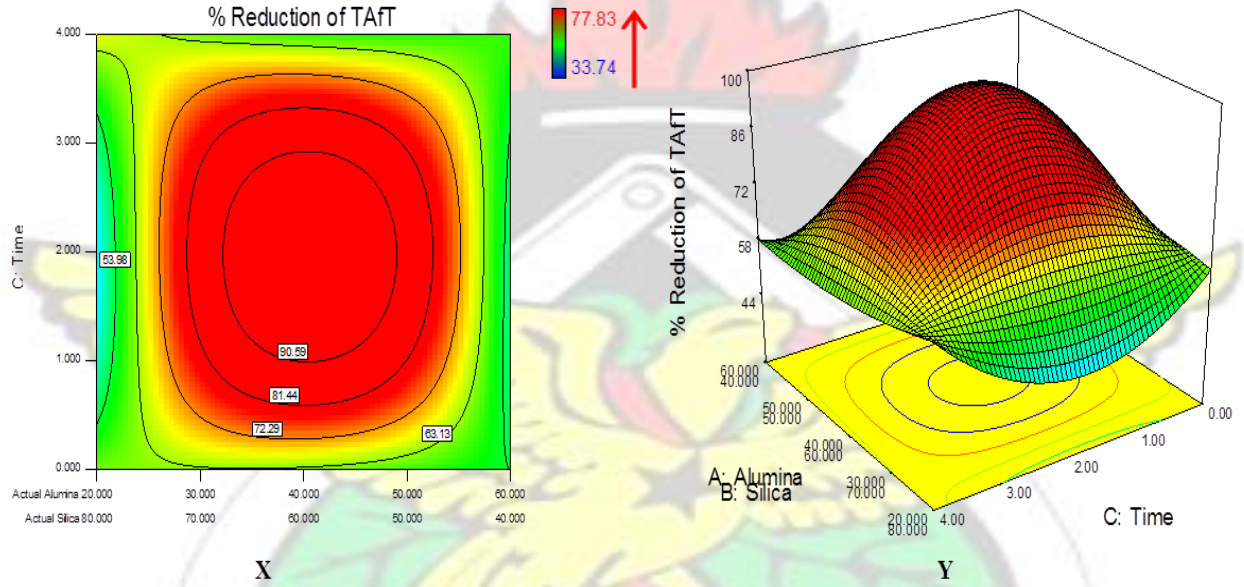

Fig 4-2: contour (X) and 3D (Y) graphs showing the interactions of the optimal conditions at a fixed temperature of 350°C.

The figure above shows that low temperature of 350 °C is favorable for aflatoxin adsorption of ASC as time increases. The large red hollow in the middle of the contour graph(X) represents the area of highest performance of ASC and the 3D mixed process graph (Y) shows a high peak also covered with red colour at the top. However, the red portion from the contour graph fades off at heating time above 3 h and also heating time below 1 h. this suggests that at the fixed temperature

of 350°C, heating time of ASC should be maximized at 3 h for optimum results but must be exceeded. This result is similar with the result from the research conducted by Wongtangtintan *et al*. (2014) which revealed that performance of Thai bentonite in aflatoxin adsorption improved when the clay was heated at a temperature range of 100-600 °C started to decline at a temperature of 700 °C.

4.5.3 Effect of Time on the Aflatoxin Adsorption Capacity of ASC

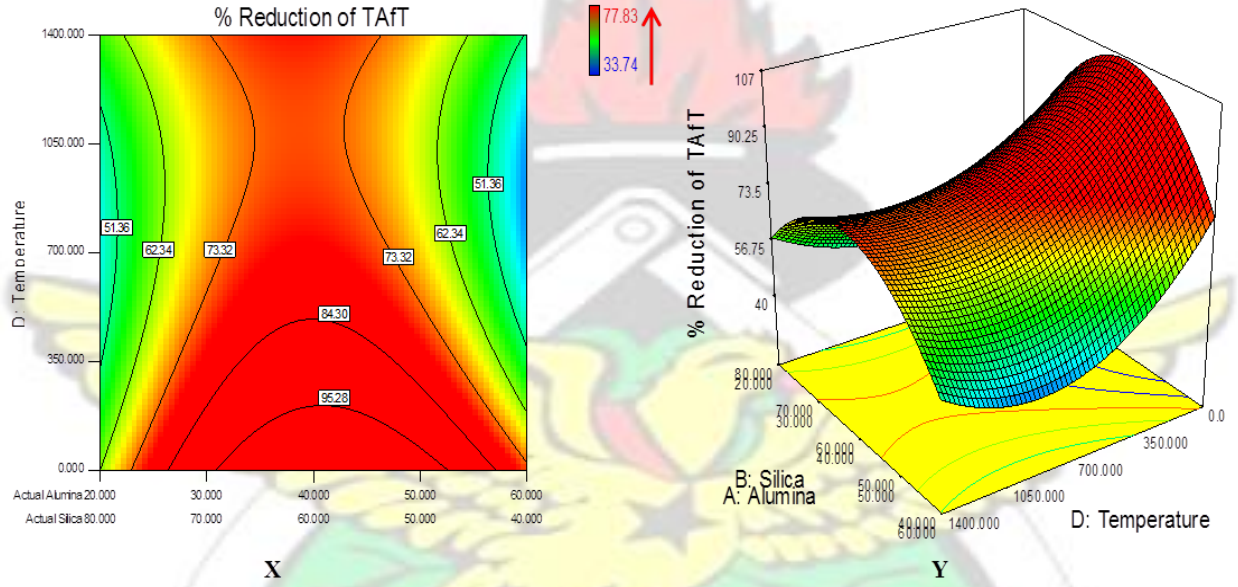

Fig 4-3: contour (X) and 3D (Y) graphs showing interaction between the optimal conditions at a fixed heating time of 3h.

4.6 FIRING OF ASC

After firing, the ASC samples in different compositions showed slight changes in their physical qualities especially with ones having more silica e.g 10:40. Alumina retained its white colour through the different firing temperatures. The colour of silica however changed from brown to peach after heating and even lighter tending to off- white colour with increase in heating

temperature. At heating temperatures of 1200 °C and 1400 °C, ASC was found to clump together making it difficult to be removed from the crucibles.

4.2 MICROGRAPHICAL ANALYSIS OF ASC

Analysis showed that the grain size of non-heated alumina (490 µm) is larger than that of nonheated silica (154 µm). However, heating both alumina and silica at 800 °C for 1 h reduced the grain length of the former and increased the grain length of the latter. This suggests that heat increases grain size of silica and a reduction in grain size of alumina. Sacks *et al*. (1997) reported that the first stage of reaction when alumina and silica are heated at temperatures within the range of 1150-1350 °C is the dissolution of alumina in the siliceous phase. Alumina diffuses more rapidly through mullite than silica. Change in the microstructure (coarsened) of alumina and silica is attributed to nucleation of mullite and growth of the grains during heating (Sacks *et al*., 1997). The micrograph of alumina:silica ratio of 30:20 is given in **fig 4-4** below.

Fig 4-4: Micrograph of ASC ratio (30Al:20si) at non-heated state(X) and when heated at 1400 °C for 2 h.

CHAPTER FIVE

CONCLUSION, LIMITATIONS AND RECOMMENDATION

5.1 CONCLUSION

This study has provided evidence to demonstrate that alumina and silica have a significant effect on *in-vitro* decontamination of aflatoxins. The second approach in this work was to investigate the effect of four independent variables which includes the percentage weight of alumina, concentration of silica, heating time and heating temperature on aflatoxin binding using a design strategy and also to predict a model for optimizing the response. Optimum conditions for the aflatoxin adsorption of ASC are combination of alumina and silica at a ratio of 32Al:68Si, heated at a temperature of 350 ^{o}C for 3 h.

5.2 LIMITATION OF THE STUDY

This study did not cover the characterization of the ASC samples using surface area analyzer and x-ray diffractors. There was no study of whether there are other health concerns involved with the use of alumina and silica in foods and feeds. Also the optimal conditions suggested by the analysis gotten from the available results were not carried out due to time limit.

5.3 RECOMMENDATION

Alumina and silica complexes utilization is a promising approach to the possibility of binding aflatoxin in foods. It therefore calls for more in depth study *in-vitro* and subsequent *in - vivo* studies to further validate the aflatoxin binding ability of these complexes. Also, interaction time between the binder and the aflatoxin stock solution should be varied and monitored in order to ascertain its

effect on the adsorptive capacity of ASC. Detailed study of the microstructure and other characteristic s of ASC should be carried out. Finally, the interaction of ASC with vitamins and other essential nutrients should be studied to ensure that the binder does not interfere with these nutrients in the body.

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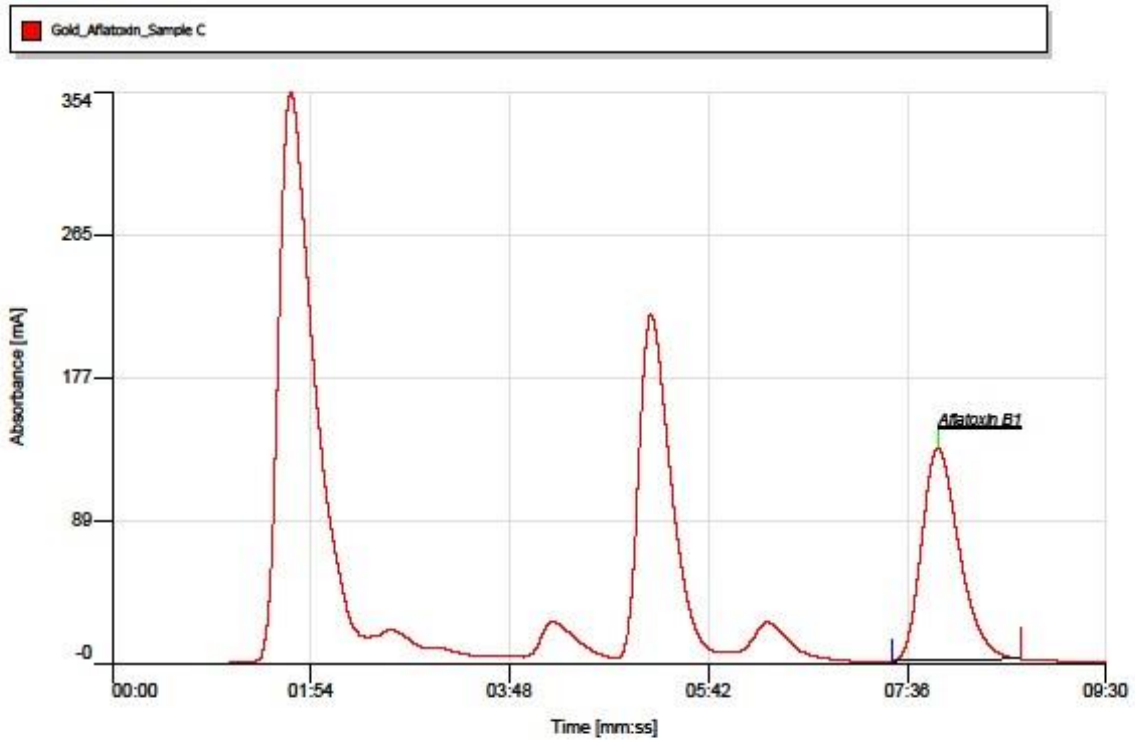
APPENDIX A

Laboratory assistant taking out samples from the furnace

APENDIX B: Chromatogram report of 15:35 ASC non-heated

Chromatogram Report

Run Time [mm:ss]	09:30.0	Sample Rate [point/s]	12.500	Readings	7125
Sample Type	Unknown	Detector Unit	A (Absorbance Units)	Detector Range	1.0000
Detector Offset	0.0000	Sample Name	Sample009/1	Method ID	16E56A8054126F73v2
Method Name	Aflatoxin_Supelco_Colum...	Amount / Final Vol.	1.000 / 1.000	ISTD Conc.	1.000

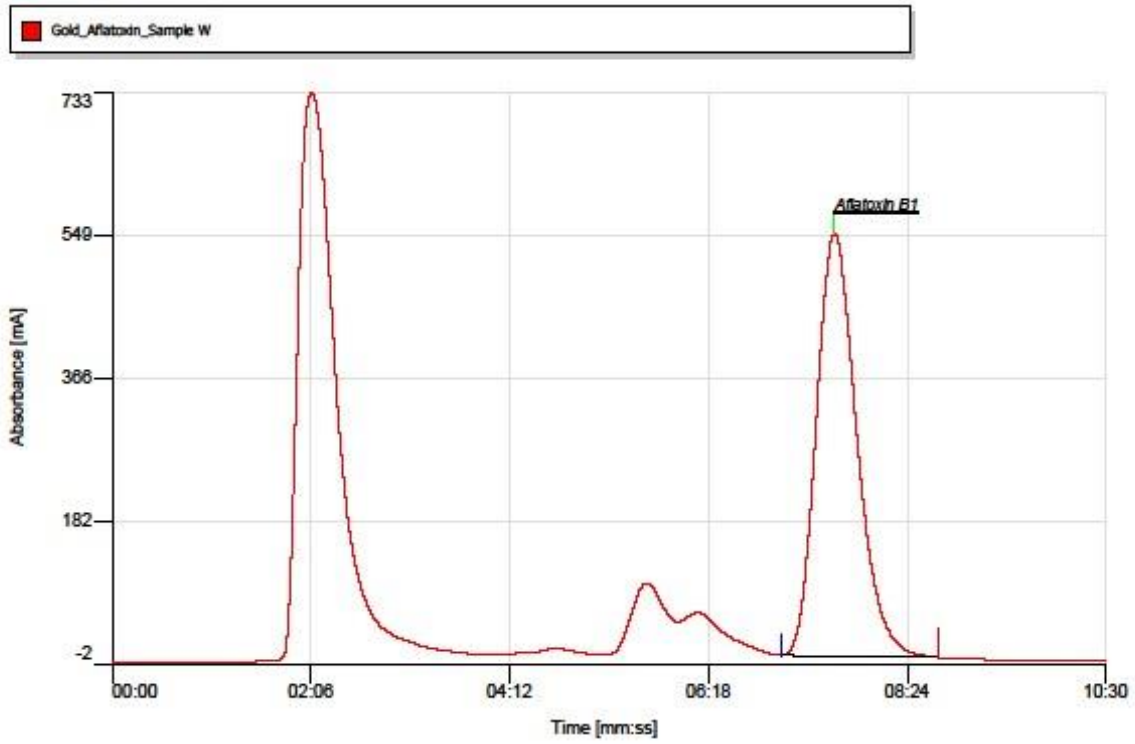


No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [ng/g]	Aflatoxin*
001	Aflatoxin B1	07:53.8	07:27.8	08:41.0	3274.9	131.8	2.7826	27.83

APPENDIX C: Chromatogram report of silica heated at 1400°C for 4hs

Chromatogram Report

Run Time [mm:ss]	10:30.0	Sample Rate [point/s]	12.500	Readings	7875
Sample Type	Unknown	Detector Unit	A (Absorbance Units)	Detector Range	1.0000
Detector Offset	0.0000	Sample Name	Sample004/1	Method ID	AS07F33A4849EBEEv8
Method Name	Aflatoxin_Supelco Sum (4....	Amount / Final Vol.	1.000 / 1.000	ISTD Conc.	1.000



No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [ng/g]	Aflatoxin*
001	Aflatoxin B1	07:38.1	07:03.9	08:43.4	16088.0	544.3	28.3015	283.01

Plate 3-1: Quartz

Plate 3-2: Silica sand

Plate 4-1: 10:40 1400°C 2H, grain length-339µm

Plate 4-2: 25:25 1200°C 2H, grain length- 220µm