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ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

CENTER FOR FOOD SCIENCE AND NUTRITION

MASTER THESIS ON:

STUDY OF AFLATOXIN AND *SALMONELLA* CONTAMINATION IN PEANUT BUTTER AND ROASTED PEANUT; THE CASE OF LOCAL AND IMPORTED PRODUCTS FROM ADDIS ABABA MARKET

By: GERESU BECHERE

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE DEGREE OF MATER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

ADVISOR: PROFESSOR NEGUSSIE RETTA

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Study of Aflatoxin and *Salmonella* Contamination in Peanut butter and Roasted Peanut; The Case of Local and Imported products from Addis Ababa Market

Msc. Thesis

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

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Declaration

I, the under signed, declare that this is my original work. It has never been submitted in any Institution and that all sources of material used for the thesis have been dully acknowledged.

Name : Geresu Bechere Tabor

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Dedication

This thesis is dedicated to all those who try to work hard towards solving problems of societies in the field of food science and nutrition.

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List of Abbreviations and Acronyms:

AFB1	= Aflatoxin B1
AFB2	= Aflatoxin B2
AFG1	= Aflatoxin G1
AFG2	= Aflatoxin G2
AOAC	= Association of Analytical Chemists
BS	= Bismuth sulfite
CDC	= Center for Disease Control
EAC	= East African Community
EC	= European Commission
EU	= European Union
FAPAS	= Food Analysis Performance Assessment Scheme
FAO	= Food and Agricultural Organization
FDA	= Food and Drug Administration
GMP	= Good manufacturing practices
HACCP	= Hazard Analyses and Critical Control Point
HBV	= Hepatitis B Virus
HCC	= Hepatocellular carcinoma
HDL	= High-Density Lipoprotein
HE	= Hektoen enteric
HKSAR	= Hong Kong Special Administrative Region
HPLC	= High Pressure Liquid Chromatogram
IARC	= International Agency for Research on Cancer
ICMSF	= International Commission on Microbiological Specifications for Foods
JECFA	= Joint FAO/WHO Expert Committee on Food Additives
KEBS	= Kenya Bureau of Standard
LDL	= Low-Density Lipoproteins
LI	= Lysin Iron
LOD	= Limit of Detection
MPI	= Ministry for Primary Industries
MSF	= Medecins Sans Frontieres

ND	= Not detected
NIST	= National Institutes of Standards and Technology
PACA	= Partnership for Aflatoxin Control in Africa
PEM	= Pathogen Environmental Monitoring Program
PPB	= Parts per billion
PPM	= parts per million
RDA	= Recommended Dietary Allowance
RUTF	= Ready to Use Therapeutic Foods
SC	= Selenite cysteine
SD	= Standard Deviation
SPG	= Southern Peanut Growers
SRM	= Standard Reference Materials
TSI	= Triple Sugar Iron Agar
TT	= Tetrathionate
UNSCN	= United Nations Standing Committee on Nutrition
WFP	= World Food Program
XLD	= Xylose lysine desoxycholate

Abstract

Aflatoxins are secondary metabolites produced by the fungi Aspergillus flavus and A. parasiticus that grow on a variety of agricultural commodities and other processed products like peanut butter. Aflatoxin B1 (AFB1) is the most toxic of all the aflatoxins produced by these fungi. Contamination of foodstuff by AFB1 is a major food safety concern in developing countries in the tropics, because of its adverse effect on human and animal health. This study was conducted in order to assess the level of aflatoxin contamination using HPLC method and detection of salmonella by conventional method in peanut butter collected from Addis Ababa supermarkets and other retailers. Analysis of aflatoxin in ready to eat roasted peanut was also part of the investigation. Study samples were collected from all margins of the Addis Ababa through dividing the city in to four directions so as to address every sampling point. Accordingly, forty eight samples of peanut butter and 10 samples of roasted peanut were randomly collected from the market, analyzed by duplicate and mean values were reported. Out of 48 samples, 45 samples were collected from local brands. The results showed that 93% of local samples of peanut butter had aflatoxins ranged from 3.92μg/kg to 547.85μg/kg, with mean total aflatoxin of 98.24μg/kg ± 111.18 SD; showing great variation of contamination among samples. Contamination range of AFB1 in peanut butter was 2.65μg/kg to 270μg/kg with mean value of 51.94 μg/kg ± 65.33 SD. All roasted peanut samples were contaminated with aflatoxin with maximum contamination of 216.48 μg/kg and minimum value was 1.86 μg/kg, having mean value of 58.78 μg/kg ±75.56 SD. The potent AFB1 was recorded up to158.83 μg/kg than other types of aflatoxins (AFB2, AFG1 and AFG2) in roasted peanut samples with mean value of 43.86 μg/kg ± 61.07 SD. Salmonella was detected in 7% (n=3) of samples of peanut butter. Oil separation was seen in 24% (n =11) of peanut butter samples. Cumulatively, 81.82% peanut butter and roasted peanut found in Addis Ababa markets were found to be unsafe as per codex maximum permissible limit for aflatoxin (≤10μg/kg). Contamination of pathogens in peanut butter was significant as far as human safety is considered. There is a need for awareness creation at all levels of the peanut value chain, especially for end consumers, campaigns to raise consumer demand for safe, high-quality food.

KEY WORDS: *Aflatoxins clean--up, peanut butter, roasted peanut, Salmonella.*

1. Introduction

Groundnut (*Arachis hypogaea L.*) is an oilseed produced all over the world and believed to have originated in Central American region from where they spread to other parts of the world (Settaluri *et al.*, 2012). The word "ground nut" is also used interchangeably with "Peanut" (SPG, 2009). It is consumed in the form of snacks such as salted nuts, confectionary sauce, cafeteria drinks, and peanut butter. Botanically, groundnut is a legume although it is widely identified as a nut and has similar nutrient profile with tree nuts (Ros, 2010). Peanut butter is made by grinding dry roasted groundnuts into a paste (Mutegi *et al.*, 2009).

The initial step in processing the groundnut is harvesting, which typically begins with the mowing of mature ground nut plants. It was once thought that aflatoxin formation only occurred at postharvest, i.e. during storage, but it is now well documented that aflatoxin production also occurs in the field prior to harvest. Risk factors for aflatoxin contamination include three or more weeks drought during pod formation, high moisture relative humidity (83%) at 30 ^{0}C and high temperature (optimum between 25-35^{0}C), rainfall at the end of the growing season that postpones harvest and prevents dry-down. In terms of storage conditions, grains with moisture levels above 9% and moderate temperatures (28 ^{0}C to 33 ^{0}C) increase the risk of aflatoxin contamination. After postharvest, the groundnut value chain starts with the farmers who either consume their produce or sell it locally at markets to rural retailers or local wholesalers who store the produce for 3–12 months. If the quality of this storage is poor, then these facilities can act as incubators for *A. flavus* proliferation and aflatoxins production and are a key point for intervention to reduce aflatoxin contamination before products reach consumers (Emmanuel, 2009).

It is possible to buy raw peanuts instead of shelled peanuts or peanut pods. The manufacturing process is briefly described hereunder. Good quality groundnut pods are sorted out and destoned before shelling them in openers. Shelled peanuts are graded according to sizes to ensure only big or bold peanuts are taken up for process. Roasting is done at around 160 ^{0}C for 40-60 minutes depending upon the moisture contents. Roasting reduces water contents to around 1% which increases the shelf life of peanuts and helps develop flavour. After roasting, peanuts are cooled and then blanched (removal of outer red skin). After blanching each peanut is inspected to remove discoloured (grey or black) nuts. Peanuts are then ground in peanut butter mill in two

stages to produce fine and creamy butter. The outlet temperature is around 65-75 ^{0}C. All ingredients like salt, sugar and stabilizers are added during this process. A scraped surface heat exchanger is used for cooling. The outlet temperature depends upon the type of stabilizer used. Peanut butter is filled in Pet Jars or metal drums as per the instructions of the buyer (Alicia, 2006).

There are many claims about the origin of peanut butter. Africans ground peanuts into stews as early as the 15th century. The Chinese have crushed peanuts into sauces for centuries. Civil war soldiers dined on "peanut porridge." In 1890, an unknown St. Louis physician supposedly encouraged the owner of a food products company to process and package ground peanut paste as a nutritious protein substitute for people with poor teeth who couldn't chew meat. The physician apparently had experimented by grinding peanuts in his hand-cranked meat grinder. Bayle mechanized the process and began selling peanut butter out of barrels for about 6 cents per pound (SPG, 2009).

Peanut butter is a good source of protein, folic acid and other B vitamins, minerals and dietary fiber. Whether you choose smooth or chunky peanut butter, the nutrient content is the same. A two-tablespoon serving of peanut butter provides 188 calories, providing at least 28% of the Recommended Dietary Allowance (RDA) of protein for children under 10, and at least 12.5% of the RDA for teens and adults (Aryana *et al*, 2000).

Peanut butter will not spoil like a perishable food, but because of its high oil content, it may develop rancidity during storage. Rancidity gives the product an "old, oily" off-flavor. It is subject to a number of microbial, chemical and physical deteriorative changes, which affect the final quality of the finished product. The shelf life is greatly dependent on the quality and conditions of the peanuts used for making the peanut butter. Deterioration of peanut butter arises from putrefaction of protein fraction caused by bacterial metabolism; darkening, which results from an interaction between sugar and protein in the product and; oxidative rancidity that develops in the unsaturated portion of oil when it is exposed to air (Flor *et al*, 2006).

The most serious problem of natural peanut butter is the tendency of the oil to separate. Oil is released during the grinding of peanuts. The improvement of emulsion stability in peanut butter

is characterized by the absence of two layers of oil and meal phase during ordinary conditions of storage, and improved texture, consistency, spread ability, flavor, color as well as nutritional value. Without stabilizers, the peanut meal settles at the bottom and forms a hard layer while the oil remains on top (Aryana *et al*, 2000).

Studies published in Argentina and China indicated that peanut butters from both countries have been shown to have higher free fatty acid percentages and peroxide values compared to U.S. peanut butter. High free-fatty acid percentages may indicate poor handling, immaturity, mold growth or other quality problems (SPG, 2009).

Peanut butter has been linked with safety issues like mycotoxin, specifically aflatoxin and salmonella. For instance, importers of Peanut butter products imported into New Zealand from Australia , have a responsibility under the Food (Importer General Requirements) Standard 2008 to ensure imports are of minimal risk for aflatoxin and *Salmonella* (PMI, 2013).

Various traditional methods are employed in the processing and preservation of the peanut and its products and some of these practices encourage fungal growth and mycotoxin production (Nautiyal, 2002). In addition to fungal growth, bacterial growth; like *Salmonella* contamination are usually encountered in peanut butter. This becomes a serious health concern since it is very difficult to remove once in food (Moss, 1996).

Groundnuts that are used as raw materials for peanut butter processing are liable to colonization by fungal molds during handling, storage and transportation, exposing them to the risk of contamination with aflatoxin (Mutegi *et al.*, 2012). Thus, Peanut butter has been monitored for aflatoxin contamination at different stages during its large-scale production starting from raw shelled peanuts up to the final product (Andrew, 2011).

If raw groundnuts are contaminated with aflatoxins, there is a high risk of exposure to the consumer through consumption of peanut butter processed from such groundnuts. Practices such as poor storage and handling within the peanut butter cottage industry could contribute to further aflatoxin contamination of peanut butter (Ndung'u *et al.*, 2013).

Mycotoxins have received considerable attention due to their significance in agricultural loss and human health. Amongst the mycotoxins that are known to cause human diseases, aflatoxins have been studied most. Aflatoxin (B1, B2, G1 and G2) belongs to a group of fungal toxins known as mycotoxins. It was discovered some 40 years ago in England following a poisoning outbreak causing 100,000 turkey deaths. Aflatoxin poses a potential threat to food safety. As aflatoxin is epidemiologically implicated as carcinogen in humans and an environmental contaminant which is widespread in nature, it poses chronic toxicity than acute toxicity (HKSAR, 2001).

Among aflatoxin groups; B1, B2, G1 and G2, Aflatoxin BI (AFB1) is a great concern in human health. AFB1 has been extensively linked to human primary liver cancer in which it acts synergistically with Hepatitis B Virus (HBV) infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (group 1 carcinogen) (Williams *et al.*, 2004).

Contamination of aflatoxin in peanut and peanut products has been reported in different countries. According to Mutegi *et al* (2010), there are many cottage industries in Nairobi that produce peanut butter. High aflatoxin levels up to 22 µg/kg in groundnut products such as roasted nuts and peanut butter have been reported in Nairobi.

Carlos *et al* (2009) summarized the data on aflatoxin contamination in peanuts and peanut products from several countries during 1982-1994, including Senegal, Mexico, United States, Philippines, India, UK and Nigeria, and concluded that aflatoxin occurrence is extremely variable worldwide, with incidences ranging from 30 to 100%, at levels up to 2,888 µg·kg-1.

To our knowledge, there is no research based data has been generated on aflatoxin contamination in products of peanuts, except for raw peanut and other commodities in Ethiopia. For instance, Fufa *et al* (1996) had made investigation and determined the level of aflatoxins contamination in shiro and ground red pepper in Addis Ababa in 1996 and found aflatoxin levels ranged from 100 to 500 µgkg-1 and 250 to 525 µgkg-1, respectively.

About 4.5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated food crops. Therefore, in order to avoid the toxicity, the levels of

aflatoxins and similar toxic compounds in foodstuff have to be monitored closely, and to be kept under control continuously. Otherwise, related health effects like acute and chronic intoxications, and even deaths, will still be an issue (Shuaib *et al.*, 2010).

On the other hand, over the last several decades, a number of outbreaks of salmonellosis have been associated with the consumption of ready-to-eat low-moisture products, including chocolate, powdered infant formula, raw almonds, toasted oats breakfast cereal, dry seasonings, paprika-seasoned potato chips, dried coconut, infant cereals and, more recently, peanut butter and children's snacks made of puffed rice and corn with a vegetable seasoning. Although *Salmonella* outbreaks from low-moisture products are relatively rare, they often impact large numbers of people. More than 200 cases were attributed to toasted oats cereal in 11 states between April and June 1998, more than 600 cases were attributed to peanut butter in 47 states between August 2006 and May 2007, and more than 500 cases have been attributed to peanut butter and peanut butter-containing products in 43 States between September 2008 and January 2009. Due to the large number of unreported cases of salmonellosis for all types of products, the actual number of cases was likely much higher (Yuhuan, 2009).

In Ethiopia, one specific case of contamination was happened in Plumpy'nut product manufactured by a company called Hilina (Company engaged in producing Ready to Use Therapeutic Foods, RUTF). The claims of contaminations were received from customers (UNICE and WFP). According to investigation report (2013) generated by the company and communicated to the customers, claim was regarding *Salmonella* detection in one batch of Plumpy'nut. According to the report, production was ceased for atleast 4 months and detailed investigation was carried out. Accordingly, contaminated cartons were segregated and rejected. Aflatoxin was also another claim in one batch of finished product showing above permissible limit of those customers (>5µg/kg), (Hilina, 2010). Hence, both reports of the company suggesting the necessity of further research on identical products like peanut butter to see contamination level of products because both RUTF and Peanut butter are peanut based products that need identical attention.

In general, there is no research based information on the safety of peanut butter in Ethiopia. However, as per information obtained from training held by Ethiopian Standard Agency for small

and large scale food processors on Food safety during March 2011, it was reflected that there was lack of knowledge and awareness among processors regarding pathogenecity and mycotoxins. Microbial safety, specifically common outbreaks like *salmonella* in the product has not been studied as most enterpreanurs are simply engaged in production of peanut butter without any safety knowledg. Hence, due to the nature of pathogenicity of *salmonella* and toxicity of the aflatoxin, health related issues should be the main concern to all researcheres of the field and government.

Previous studies have not investigated the status of aflatoxin and pathogenic contamination in peanut butter from cottage industry of Addis Ababa. Hence, more assessments on foodstuff are needed to identify public health strategies that could be integrated with current agricultural and processing approaches to reduce possible problems associated with the consumption of aflatoxin and pathogen contaminated food in Ethiopia.

1.1. Statement of the problem

Now days, Processed groundnut is widely consumed by most people (wide age group) in diversified ways including peanut butter which is used as spread for bread or biscuits, in cookies, sandwiches, candies and frostings or icings. Recently, peanut butters are also drunk at cafeterias and restaurants as peanut tea. According to Ethiopian Food Corporation (1998), the use of such processed foods in Ethiopia is increasing. So, it is possible to observe that consumption of peanut butter has been increasing and hence different peanut butter processers with different market brands have been emerged in the market at alarming rate. The trend of life of society in cities has increased the demand of such ready to eat food like peanut butter.

The demand for the product is met through local production and import. According to the report of Southern Nations and National Peoples' Regional State Investement Bureau (2011), during the period 2000 – 2006 a total of 53,311 kg butter have been imported from abroad with an average annual import of 4,544 kg. Moreover, the required demand of peanut butter for local market during 2010 was 16,236kg and forecasted up to 19,337kg, 20,497kg, 21,727kg and 32,670kg for the year 2013, 2014, 2015 and 2022, respectively. According to retailers in the field, the local supply of peanut butter is at least twice the imported one. Therefore, by considering the various factors, the demand for peanut butter is projected by employing an average annual growth rate of 6%. However, regardless of the increasing demand of the product, the safet of the product has got less attension.

As per different information from media, patients with liver problems and Hepatisis B virsus are growing in Ethiopia. According to Ramesh, *et al.*, (2003), epidemiological studies carried out in several parts of Africa and Asia indicate a correlation between exposure to aflatoxins and primary liver cancer. The risks associated with exposure to aflatoxins are enhanced by simultaneous exposure to hepatitis B virus and possibly hepatitis C viruses. Recent studies carried out in West African countries including; Benin, Gambia, and Togo indicate chronic exposure of population groups and fetuses to dietary aflatoxins. Children exposed to aflatoxin may become stunted, underweight, and more susceptible to infectious disease in childhood later life (Ramesh *et al.* 2003). In Ethiopina too, dietary exposure to aflatoxins is considered among the possible causes for high level of chronic liver diseases (Fufa,1996). In a research conducted by Chala (1996) on aflatoxin content of ground nut from eastern Ethiopia, heavy contamination of aflatoxin is reported (i.e. 11,900 μg/kg). The amount reported by Chala is within the level of contamination that had caused sudden fatalities in India which was between 6.25 -15.6 ppm (Agag, 2004). Products which use this crop as recipe, such as peanut butter, could pose a danger to consumers.

On the other hand, due to poor post harvest handling of peanut and lack of awareness, microbial contaminations are also expected as processers may not process the peanut butters under good hygienic environment and right temperature. As a result, salmonellosis would possibly be an issue in the peanut butter, as reported in other countries.

Plenty of researches have revealed that *Salmonella* contamination is a common outbreak in different world countries. Accordingly, peanut butter produced in Ethiopia has not been assessed for *Salmonella* contamination. The reason for existence of *Salmonella* in peanut butter could be due to the presence of cross contamination risks that may arise from premises of processors. The problem is also agravated due to the fact that no national standards and controlling mechanisms for all processers of peanut butter and ready to eat roasted peanut.

In addition,the peanut butters imported from different countries have not been assessed regarding aflatoxin and microbial contaminations.

However, regarding both types of contaminations in Ethiopia, specific case of Hilina (Company engaged in Producing RUTF) can be raised here as an example. Similar peanut based product like peanut butter known by the brand name "Plumpy'nut" (RUTF) has showed contamination of aflatoxin (12μg/kg) and *Salmonella* since few years ago in differen two batches of the product

(Hilina, 2010; Hilina 2013). Likewise other similar cases may be appeared in many peanut butter processors.

Therefore different investigations have been made on raw peanut. However, no research based data is available regarding aflatoxin and *Salmonella* contamination in peanut butter in our country. Processors thought that bad and infested kernels of peanut can be used for peanut butter production because quality criteria for such product are not clear for most of the processors and consumers. This undestanding will result in production of highly contaminated product with aflatoxin. Local peanut butter has been produced traditionally on a small scale and as such has been given little or no attention on the microbiological quality and mycotoxin safety. As a result, the safety of Ethiopian processed peanut butter is lacking in literature.

Thus, this research aimed at seeing the degree of aflatoxin and *Salmonella* contamination in commercially processed peanut butter in Addis Ababa markets. Ready to eat roasted peanut was also part of this investigation.

1.2. Research Question

- To what extent are commercially processed peanut butter and roasted peanut contaminated by aflatoxin?
- Is there any contamination of *Salmonella* in processed peanut butter?
- Do standards of aflatoxin and *salmonella* exist in Ethiopia?

1.3. Significance of the study

Understanding and knowledge of food safety among most societal members are limited. Lower level of educational background within the community and lack of researches on food safety would lower the awareness of the issue. Whereas, the existence of food safety related risks including aflatoxin and pathogens more commonly *Salmonella* have been far and wide in Ethiopia. This study, therefore, will help consumers to have an idea about aflatoxin and pathogens safety and hence primarily be benefited. Processors will develope knowledge about the safety of peanut butter and hence this study will urge them to develope good hygienic and manufacturing practice. Government regulatory bodies can use the study result for establishing

different local standards. Moreover, the study will be a baseline data for the next coming researches; including on peanut and peanut products and other food related risk assessment. It helps consumers in alerting on food safety and quality matters as well.

1.4. Objectives:

General Objectives:

To determine the status of aflatoxin and *Salmonella* contamination in local and imported peanut butter and ready to eat roasted peanut traded in Addis Ababa markets.

Specific Objectives

- To assess the aflatoxin content of peanut butter from different brands found in Addis Ababa market.
- To analyze aflatoxin content of ready to eat roasted peanut collected from different retailers
- To analyze *Salmonella* in peanut butter from different brands found in Addis Ababa market.
- To determine the quality of peanut butter intems of oil separation.
- To cross check the availability of Ethiopia standards on Aflatoxin and *Salmonella*.

2. Literature Review

2.1. Overview of Peanut and Peanut butter

Peanut (Groundnut) is one of the most important crops in different parts of the world because it is not only a useful crop for rotation with corn and other cereals, but also a cash crop (Geleta *et al.*, 2007). Peanut occupies an important position in the developing countries; the major peanut producing countries are India, China and the United States (Cynthia, 2009).

The lowland areas of Ethiopia have considerable potential for increased oil crop production including groundnut. The estimated production area and yield of groundnut in Ethiopia in 2010/2011 cropping season were 49,603 hectares and 716,068 quintals, respectively, and the largest groundnut production areas are found in Oromiya (32967.8 ha), Benshangul-Gumuz (9968.73 ha), SNNPR (635.04 ha) and in Amhara (344.57 ha) regional states (CSA, 2011). Somalia and Gambela regional states also produce a considerable amount of groundnuts (Alemayehu et al., 2014).

Research result showed that groundnut farmers can produce groundnut yields of 2000 kg/ha or more, but the national average yield produced by the farmers in Ethiopia is considerably low, 1200kg/ha. Similarly, Central Statistics Agency of Ethiopia (2009) survey report revealed that 1123 kg/ha average yield of groundnut per year (Gezahagn, 2013).

More than 300 different varieties of peanuts are grown worldwide, which include Virginia, Valencia, Georgia runner, Tennessee red, Tennessee white and many others. They are usually consumed after roasting or boiling, and also processed into different forms such as peanut butter, candy, chocolates, cakes, and others. Peanuts vary in color from red to brown and are usually coarse in their appearance (Settaluri *et al.*, 2012).

The nutritional importance of peanuts is due to the energy and growth supplementing constituents present in them. These include 49 % fat by weight (of which about 14 % are saturated fatty acids, 51 % are monounsaturated fatty acids (mostly oleic acid) and 33 % are polyunsaturated fatty acids (mostly linoleic acid), 16 % carbohydrates, 9 % fibre and 25 % protein along with, vitamins, minerals, some organic acids and purines (Odu 2012).

It is estimated that as much as 30% of the population from many countries in the world is suffering from malnutrition (FAO, 2000). Peanuts, which are a rich source of protein and essential amino acids, can help in preventing malnutrition. Moreover, peanuts contain lipids and carbohydrates which are energy rich compounds, capable of complementing the basic energy demands of the human body (Settaluri *et al*, 2012).

Peanut butter is a food paste made primarily from ground dry roasted peanut and is popular in the Philippines, North America, the Netherlands and the United Kingdom. It is mainly used as a sandwich spread, sometimes in combination (peanut butter and jelly sandwich) (Odu 2012).

Composition of peanut butter may vary and it may contain added palm oil or partially hydrogenated vegetable oil. For peanut butter manufactured from roasted peanuts only, the fatty acid composition depicted above applies. Peanut butter, which is not manufactured exclusively from roasted peanuts is not sufficiently characterized in relation to the claimed effect because of its unknown fatty acid composition. Peanut butter manufactured exclusively from roasted peanuts, which are the subject of the health claim, are sufficiently characterized in relation to the claimed effect. The claimed effect is "helps achieve normal cholesterol levels by reducing blood total Low-Density Lipoproteins (LDL)-cholesterol and thereby promoting heart health". LDL carries cholesterol from the liver to peripheral tissues, including the arteries. Elevated LDL-cholesterol, by convention >160 mg/dL (>4.1 mmol/L), may compromise the normal structure and function of the arteries (EFSA, 2011).

New research shows that a higher unsaturated-fat, lower-carbohydrate, peanut and peanut butter diet for weight loss reduces the risk of cardiovascular disease by 14% compared to baseline. The peanut rich, higher unsaturated-fat diet resulted in favorable heart-health benefits and also had the added cardiovascular benefit of maintaining "good" high-density lipoprotein (HDL) cholesterol and lowering triglyceride levels. In addition, people who ate the moderate-fat diet were able to favorably affect certain cholesterol ratios, which are increasingly seen by health professionals as a more comprehensive assessment of heart disease risk. Thus, a balanced diet with peanuts and peanut butter can help with weight loss, while improving heart health and

eating satisfaction! The key is to replace foods high in saturated fat with peanuts and peanut butter (Pelkman, 2004).

However, parallel to the broad advantages of peanut and peanut butter, there is a concern related to peanut allergy. Peanut allergy affects approximately 1.6% of school age children. Peanut is one of the few allergens that are capable of causing life-threatening reactions. Current treatment of food allergies includes avoidance of the trigger food and treatment of a severe reaction with epinephrine (Watson *et al*, 2013). In other study of Settaluri (2012), It was found in 0.4% to 0.6% of persons, particularly in children, consumption of peanuts in any form lead to allergic reactions, some severe. These may develop in the childhood or could be due to a family history of peanut allergy

In addition, peanut butter has been frequently associated with mycotoxins and food illness in which initial contamination is traceable to food handlers. Numerous epidemiological reports and studies have implicated foods of ready to eat origin as the major vehicles associated with mycotoxins and illness caused by food- borne pathogens (Odu 2012).

2.2. Toxicological Point of View

2.2.1. Mycotoxins

Mycotoxins are secondary metabolites produced by microscopic filamentous fungi, which can develop on food crops (groundnut, maize, wheat, etc.) and in some cases on commodities of animal origin (meat products, sausages). Mycotoxins are harmful to vertebrates when they are absorbed through ingestion, inhalation, or dermal absorption. It has been shown that ingestion of contaminated food/feed is the main source of mycotoxin exposure to both humans and animals (Ajoy *et al*, 2010; Ilunga, 2012).

Mycotoxins are mainly produced by fungal species belonging to the genera *Aspergillus, Penicillium* and *Fusarium* which are ubiquitous in the environment (Klich, 2007). Under varying conditions, also depending on the species and strain, specific fungi produce a particular

mycotoxin, specific fungi produce many mycotoxins or again a specific mycotoxin is produced by a variety of fungi (Viljoen, 2003).

The word mycotoxin comes from the Greek word 'mykes', meaning mould, and 'toxicum' meaning poison, and the diseases caused by them are called mycotoxicoses. Historically, mycotoxins have probably been present in food and feed since early in the history of humanity and some of their effects have been recognized for centuries (Viljoen, 2003).

There have been many mycotoxin-related outbreaks in the past century which have led to numerous deaths, e.g. 'yellow rice disease' in Japan and Alimentary Toxic Aleukia which killed many Russian people. In the 1930s, another 'Russian' disease linked to mycotoxin exposure in the Ukraine led to the death of many horses (Ilunga, 2012).

Scientists estimate that there are 300 to 400 mycotoxins presently identified with more being isolated as new techniques and processes evolve. But, the scientific interest has thus far been concentrated simply on more or less 10 compounds presenting a known toxicological impact on human and animal health. A list of mycotoxins significantly impacting agricultural commodities would include aflatoxins, zearalenone and trichothecenes, ochratoxin and fumonisinsm (Ajoy *et al.*, 2010; Wu *et al.*, 2011).

But, the scientific interest in mycotoxins and their implications for human and animal health began in the 1960s when 100 000 young turkey poultry died in England from a seemingly new disease, *'Turkey "X" disease'*, followed suddenly by the death of as many as 5,000 partridge and pheasants poultry on a single farm, 14,000 ducklings on another farm and severe losses of ducklings, reported from as far as Kenya and Uganda; these events triggered renewed attention being paid to the mystery killer disease (Ajoy *et al.*, 2010; Ilunga, 2012).

Literature review by Kumar *et al.* (2008) on the incidence of mycotoxins in some commercially important agricultural commodities concluded that high risk commodities for mycotoxin contamination were corn and groundnut. Similar conclusion was also drawn by Wagacha *et al.* (2008) from the African perspective too, in which aflatoxins widely occured in groundnuts.

2.2.2. Aflatoxins

2.2.2.1. Natural Occurrence

Aflatoxins are mycotoxins that have been well-known since the outbreak of *'Turkey "X" disease'* in England, first isolated and characterized from *Aspergillus flavus*; this mould is a common contaminant of poorly stored grains (Klich, 2007).

In crops under severe drought stress, peanuts begin to dry in the ground, and under these conditions luxuriant growth of *A. flavus* can occur, with high aflatoxin production (IARC, 1998).

Aflatoxin is the secondary metabolite produced by specific strains of *Aspergillus*. These species contaminate various agricultural commodities either before harvest or at post-harvest stages under favorable conditions of temperature and humidity. *Aspergillus* is a large genus of mould which grows at an optimal range of temperature of 28-33 ^{0}C and at the water activity of about 0.83-0.97. The aflatoxigenic moulds are principally found in soils and decaying vegetation (HKSAR, 2001).

A. flavus, *A. parasiticus* and *A. nomius* occur in warmer parts of the world such as tropical region where temperature and moisture are high. They have a higher affinity of growth in nuts and oilseeds. Countries in latitudes between 40°N and 40°S which includes all of Africa are susceptible to aflatoxin contamination (PACA, 2012).

2.2.2.2. Characteristics of Aflatoxin

Aflatoxin is classified into a number of subtypes. However, the most important ones are B1, B2, G1 and G2, distinguished by their fluorescent colour under ultraviolet light. In addition, the aflatoxin B, present in contaminated peanut meal fed to cows, is metabolized by the cow and excreted in the milk as aflatoxin Ml-also a potent liver toxin. AFB1 is usually found in the highest concentration followed by AFG1, AFB2 and AFG2. (Yousif *et al*, 2010).

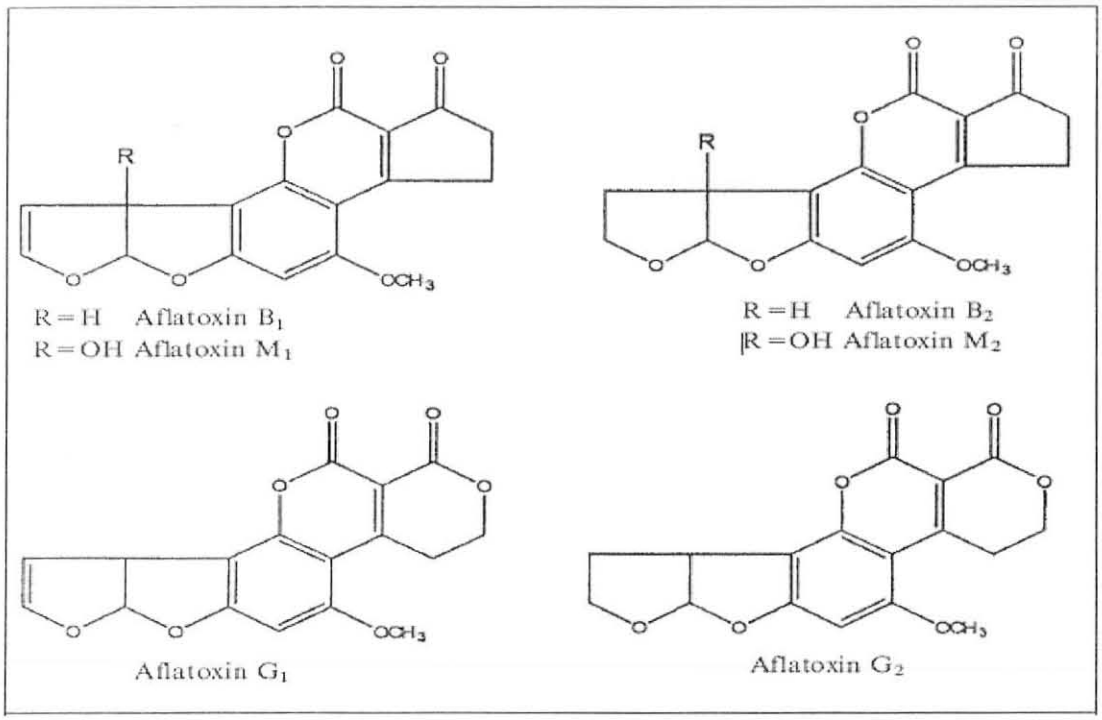

Figure 1: Structures of naturally occurring aflatoxins

Source: (IARC, 1993)

Unlike *Aspergillus*, which often looks greenish to the naked eyes, aflatoxins are odourless, tasteless and colourless. Chemically, they are stable in food and resistant to degradation under normal cooking procedures. It is difficult to eliminate aflatoxin once it is produced. Accumulation of aflatoxin is dependent upon weather conditions. Before harvest, the risk for the development of aflatoxin is greatest during major droughts. When soil moisture is below normal and temperatures are high, the number of *Aspergillus* spores in the air increases. These spores infect crops through areas of damage caused by insects, and inclement weather. Once crops are infected, plant stress occurs, the production of aflatoxin is favoured (Cynthia, 2009).

The four compounds are distinguished on the color of their fluorescence under long-wave ultraviolet illumination (B = blue, G = green), with the subscripts relating to their relative chromatographic mobility. Aflatoxins are slightly soluble in water (10-30 (μg/ml), insoluble in non-polar solvents, and freely soluble in moderately polar organic solvents (e.g., chloroform and methanol). The lactone ring is susceptible to alkaline hydrolysis; aflatoxins are also degraded by ammonia or sodium hypochlorite. The melting points of aflatoxins are very high ranging

between 237-289 °C, so aflatoxins can not readily be degraded under normal home cooking conditions such as boiling and frying (approx. 150 °C) (Omer, 2001).

During post-harvest stage, proliferation of aflatoxin can be exacerbated in susceptible commodities under storage conditions such as hot and humid storage environment (HKSAR, 2001).

Aflatoxin due to the invasion of *Aspergillus flavus* of the peanut (groundnut) pod is a serious problem in the international peanut market and has seriously hampered the export business of the developing countries (Cynthia, 2009).

The presence of aflatoxins in peanut products in Africa has been recognized as a potential danger to human health (WHO, 2006).

2.2.2.3. Health Implication / Toxicity of aflatoxins

Human exposure to aflatoxin is principally through ingestion of contaminated foods. Inhalation of the toxins may also occur occasionally due to the occupational exposure (HKSAR, 2001).

Aflatoxin contamination of key staples maize, groundnuts and sorghum occurs above safe levels in many African countries. Prevalence data from Africa suggests that aflatoxin contamination in maize, groundnuts and sorghum is higher than the European Union aflatoxin standard (4 μg/kg) and that of USA (20 μg/kg) in many countries. However, even aflatoxin exposure at low levels can result in measurable human health impacts (PACA, 2012).

A study conducted in India indicated that 21% of the contaminated groundnut samples available in the Indian market were not suitable for human consumption as they contained aflatoxin B1 above the Indian permissible limit of 30μg/Kg (Bhat *et al.*, 1997).
Aflatoxins are considered to be genotoxic carcinogens and have been shown to cause cancer in laboratory animals by reacting with DNA. In humans, aflatoxins have been linked to liver cancer

in a number of developing countries, where it is common for some foods that are an important part of the diet to contain high levels of aflatoxins (Judy, 1998).

Aflatoxins are reported to be toxic to a wide range of organisms, from bacteria to vertebrates and primates. Wide differences in toxicity have been detected among the various chemical members of the aflatoxin family. The relative potency of the four aflatoxin types as assessed in ducklings is, AFB1 > AFG1 > AFB2> AFG2 with LD50 values of 0.36, 0.78, 1.70, and 3.44 mg/kg, respectively (Leo 2013).

However, data on clinical aflatoxicosis in humans is limited although evidence exists for aflatoxicoses of human populations in many areas of the world. Evidence of acute aflatoxin toxicity has been reported from Taiwan and Uganda; it is characterized by vomiting, abdominal pain, pulmonary oedema, and fatty infiltration and necrosis of the liver, but the intake level was not reported. An outbreak of aflatoxin poisoning as a result of the consumption of a heavily moldy corn (6-16 mg aflatoxin/kg), was also described in Western India (Omer, 2001)

Furthermore, according to Yousif *et al.* (2010) and William *et al.* (2004), among the naturally occurring aflatoxins (B1, B2, G1 and G2), aflatoxin B1 is the most important for humans and animals by its frequency and toxic compound, and is responsible for renal complications, immunological and cancérigénique.

According to Yousif *et al.* (2010), aflatoxin contamination in Sudanese groundnut and groundnut products and found that percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively. They confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2.

Omer *et al.* (1998) carried out a comparison of aflatoxin contamination of peanut products in two areas of Sudan. On the basis of 'clinical experience and Khartoum hospital records', the authors suspected that incidence of hepatocellular carcinoma (HCC) or primary liver cancer was substantially higher in western Sudan than in central Sudan. The study was carried out in 1995 and involved selection of peanut butter samples from local markets using a staged sampling approach to identify markets in the two study areas. Samples were characterized as to how they

had been stored and were analysed for aflatoxin B1 by HPLC. Mean aflatoxin B1 levels were much higher in 'high-risk' western Sudan (87.4 ± 197.3 μg/kg) than in central Sudan (8.5 ± 6.8 μg/kg).

In other study of Omer *et al.* (2001) highlighted that in a Sudanese case–control study of liver cancer, a relationship was found between reported ingestion of peanut butter and liver cancer in a region with high aflatoxin contamination of peanuts, but no such relationship in a region with low contamination of Peanuts.

Ramjee *et al.* (1992) and Williams *et al.* (2004), pointed exposure to aflatoxin as a causal or aggravating factor for kwashiorkor in African children and suggested that chronic exposure to aflatoxins was associated with impaired immunity, malnutrition and liver cancer which is the third most common cause of death from cancer in Africa.

Some of the common effects of chronic aflatoxicosis include impaired food conversion, slower rates of growth, and a decrease in absorption of various micronutrients (Jolly *et al.*, 2007). Aflatoxin contamination of groundnuts therefore poses a risk to human health and has been identified as a major constraint to trade in Africa (Ndung'u *et al.*, 2013). Acute and chronic toxicity of aflatoxins have been demonstrated in several animal species including birds, fish, domestic animals and primates. Cases of human acute aflatoxicosis have been recently been reported in Kenya where the largest and the most severe documented aflatoxicosis poisoning outbreak resulted in the level as high as 8,000 μg/kg in Kenya in 2004, causing 125 deaths out of 317 case-patients (Azziz, 2005; Wagacha *et al.*, 2008). In India where 106 deaths were also reported out of 400 cases and other places in Asia such as Malaysia (Andrew, 2011).

One of the first major documented reports of aflatoxins in humans occurred in western India in 1974 where 397 persons were affected and 108 persons died. More than 150 villages were involved (Lewis *et al.*, 2005).

There is also mounting evidence implicating aflatoxins as an important factor in infant under-nutrition and impaired growth in children under five years old. This has been attributed to aflatoxin suppressing the immune system and absorption of micronutrients (Andrew, 2011).

In Benin and Togo, children in high aflatoxin exposure zones were found to gain 22 percent less height than children in low-exposure zones. Childhood exposure to aflatoxin in the Gambia was also associated with immune suppression. Growth and immune impairment are critical in predisposing children to the infections that result in the high morbidity and mortality in African populations (WHO, 2005). To this end, there was no similar research found in Ethiopia.
In 1993 the International Agency for Research on Cancer (IARC) classified AFB1 and mixtures of aflatoxins as Group 1 carcinogens, i.e. substances that can cause cancer in humans (Andrew 2011).

2.2.2.4. Review of related works done on Aflatoxins contamination around the world.

In a recent study by Ndung'u *et al*, (2013), 37% of groundnuts and their products including peanut butter and peanut flour sampled from Nairobi, Nyanza and Western Kenya did not meet the 10 μg/kg total aflatoxin limit set by the Kenya Bureau of Standards. Peanut butter is one of the main products of groundnut processing which is used as a sauce or spread in many households in Kenya (Campos-Mondragón *et al*., 2009). Most of the peanut butter consumed in Nairobi is produced in the cottage industry. High aflatoxin levels up to 22/kg in groundnut products such as roasted nuts and peanut butter have been reported in Nairobi (Mutegi *et al*., 2010).

Another report of Mutegi *et al.* (2013) showed that, independent of provenance, 69% of peanut butter products and 75% of sampled split nuts had aflatoxin levels exceeding 10 mg/kg. Only 4% of the peanuts and peanut products would have been accepted under the KEBS regulation but rejected under the EU regulations. However, 37% of the sampled peanuts would have been declared unfit for human consumption under the KEBS (>10 mg/kg). In three province of Kenya contamination level of aflatoxin varies. In Nairobi, Nyanza and Western Kenya percentage of aflatoxin contamination in roasted peanut greater than 4μg/kg were 56.2%, 24.7% and 39.5%, respectively.

Research conducted in Sudan showed that AFB1 concentrations in peanut butter was between <1 μg/kg (detection limit is 1 μg/kg) and 781 μg/kg. Sixty-three percent of the samples from West Sudan were above the permissible level of 10 μg/kg, and 50% of the samples from Central Sudan exceeded this level (Omer, 2001).

A Korean survey of different nuts and their products marketed in South Korea showed that 9 out of 85 samples including peanuts, peanut butter, and pistachios were contaminated with aflatoxins (10.6% of incidence), The most contaminated nut was peanut (roasted) with values ranging from 2.00–28.24 μg/kg and a mean of 10.67 ± 12.30 μg/kg for total aflatoxins (7.97 ± 7.75 μg/kg for aflatoxin B1). (Mehrdad *et al.*, 2011).

In a study by Ismail *et al.* (2010), from about 196 nuts and their products in Malaysia, 16.3% of the products showed contamination between 17.2 to 350 μg/kg. The highest aflatoxin content,841ppb was recorded in ground nut paste in Ghana during 2010 (RASFF, 2011).

According to Freitas *et al.* (1998) in Brasil, aflatoxin B1 was detected in Peanuts and its products including roasted peanut and peanut butter upto max. 1789ppb and from total of 80 samples, 41 happened to be positive. Similarily, Zhang *et al.* (1996); indicated that in Asia more specifically, in China, Malaysia and Philippines, 235 of 594 samples (Peanut butter and roasted peanut) were contaminated with Aflatoxin B1 at levels ranging from 1 to 244μg/kg.

According to Carlos *et al.* (2009), the occurrence of aflatoxins in Brazilian foodstuff has been frequently reported, and is a particular problem in peanuts, with up to 52% of the samples analysed being positive for aflatoxins. In the state of São Paulo, contamination of unprocessed peanuts and peanut products by AFB1 was reported in the period 1988-1999 with mean levels ranging from 51 to 420 μg·kg-1. Although the levels of aflatoxins are presumed to be lower in processed foods, due to quality control systems applied by the industry especially after the implementation of the tolerance limit in 2002.

The study of Carlos *et al.* (2009) ephesised that after the implementation of tolerance limit in Brazilian foodstuff, the incidence of aflatoxin in both processed and un processed peanut has

been decreased ; because, on his finding, the incidence of aflatoxin in unprocessed peanut was signifcantly decreased from 52% to 39.6% of samples analyzed with mean concentration of total aflatoxins (12.88 ± 2.42 μg·kg-1) and no sample of salty roasted peanut had aflatoxin concentrations above the tolerance limit adopted in Brazil. In Brazil, good manufacturing practices (GMP) have become compulsory for peanut industries since 2003, when the National Health Surveillance Agency issued the regulation. Hence the author concluded that , the decrease in aflatoxin in Brazilian peanut products in recent years may be a consequence of specific changes in industries that have implemented GMP.

Report from Shami *et al.* (2010) showed that twenty two samples (18.33%) of the one hundred and twenty samples of peanut and peanut butter studied in Sudan showed similar contamination level of aflatoxin B1 with level of aflatoxin B1 studied in Botswana, in a range of (0.8 – 16.00 μg/ Kg) for the raw shelled peanut samples and for peanut butter were (3.2 – 16.00 μg/ Kg). Moreover, Shami *et al.* (2000) and Ali *et al.* (1999) had declared almost similar occurence of AFB1 in peanut butter upto (53.33%) and 50% in their respective survey reports.

In recent study conducted in Bamako (capital cityof Mali) and Kita (other market area of Mali) *t*o assess the occurrence of aflatoxins in peanut butter indicated that 90% of peanut butter samples from Bamako and Kati contain detectable aflatoxin B1 in a range of 0μg/kg - 189.29μg/kg. The mean level of aflatonin B1 in peanut betters analyzed was 4μg/kg in Bamako and 34.086μg/kg in Kita. Referring to the European standard which is 5 μg/kg, peanut butters purchased in Bamako are good in terms of aflatoxin content, and product from Kita were heavily contaminated by the aflatoxin B1. These high contaminated peanuts butters made in home entered the local market distribution system, resulting in widespread aflatoxin contamination (Keita *et al.*, 2013).

The study conducted in Papua New Guinea to assess the prevalence of AFB1 contamination in some foodstuff revealed that 13 samples of Peanut butter showed aflatoxin contamination with mean and median values of 3.8μg/kg and 1.9 respectively having contamination range between below detection to 16.7μg/kg. The AFB1 levels in 53.8% of the samples were below 2.0μg/kg and 92.3% were below 10.0μg/kg and 15.0μg/kg. None of the peanut butter samples were highly

contaminated with AFB1. The dried roasted peanut group has mean AFB1 level (6.1μg/kg) with the range of (0.0 – 29.3μg/kg), (Chilaka, 2012).

The UK Committee on toxicity of chemicals in food, consumer products and the environment has, since1981, recommended that aflatoxin concentrations in food should be reduced to the lowest levels technologically achievable. The European Commission's Scientific Committee for Food also concluded that there was a need to reduce aflatoxin exposure to the lowest level achievable (Judy, 1998).

After peanuts are shelled, several physical procedures such as colour sorting, density flotation, blanching and roasting are routinely used by processors to reduce aflatoxin levels by 99%. The colour sorting process was developed originally to reject commercially unacceptable discoloured nuts, but as fungal growth is a prime cause of discolouration, the process is also an effective non-destructive means of removing most nuts containing aflatoxins. In crops under severe drought stress, peanuts begin to dry in the ground, and under these conditions luxuriant growth of *A. flavus* can occur, with high aflatoxin production. In this case, blanching removes skins, and roasting to increase discoloration which permits colour sorting to be carried out. Roasted peanuts must be sold under inert gas atmospheres to suppress development of rancidity (López-García *et al.*, 1999).

The precence of aflatoxin in food is , however, often over looked due to public ignorance about their existence. Apart from economic issues, ethical considerations also play a role during the manufacturing process of food products using heavily contaminated commodities. Instead of excluding these from the food process, unscrupulous manufacturers sometimes dilute contaminated agricultural products such as peanuts with good- quality products to an acceptable level below the regualotry level or market term as such when no regulatory measures are enforced (Bhat *et al.*, 2010).

Study conducted in Ethiopia showed that from total 52 raw peanut samples analyzed for aflatoxins presence, the result showed that 38 samples found to be contaminated with aflatoxins in a range of concentration between 0.57μg/kg and 447.02μg/kg, from which FB1 was

predominantly found upto 324.34μg/kg (73.07%). The finding categorized the results against international standards of European Union (4μg/kg) and FDA (20μg/kg), and accordingly, 83.33 % and 91.67 % of newly harvest peanut collected from three localities were below 4 ppb and 20 ppb respectively. Contrarily, from samples of peanut stored for one year, only 4 % and 52 % were below the up mentioned international standards, due to handling problem (Eshetu, 2010).

According to Ali *et al.* (1999), when the initial content of aflatoxin was high in the raw shelled peanut, a high level of aflatoxin contamination can be expected in its final products such as peanut candy and peanut butter. On the other hand, the low level of aflatoxin contamination in the peanut products has always been associated with the use of high quality raw materials (raw shelled peanut) that contain an acceptable low initial level of aflatoxin. Besides, some literatures reflected that various peanut processing techniques, such as shelling, drying under sunlight, boiling with salty water, and roasting, were also found to be useful in reducing the aflatoxin content in the products; which of course needs more detailed invesigation and this paper did not focus on recommended statements.

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To our local experience, most local processors of peanut butter have no technical knowledge on the quality of peanut. They simply produce and sell their product without any due attension on their product quality. Absence of local standards on aflatoxin, low support and control of regulatory bodies on peanut and peanut product manufactuers aggravates the problem.

2.2.2.5. Economic Impact of Aflatoxins

The economic impact of aflatoxins was derived directly from crop, livestock losses, and, indirectly, from the cost of regulatory programs designed to reduce risks to animal and human health. The Food and Agriculture Organization (FAO) estimates that 25 % of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins (WHO, 2006).

Aflatoxins cause economic and trade problems at almost every stage of marketing of groundnut especially during export. Earlier reports indicated that over a decade, the export of groundnut productions from India has declined from 550 metric tons (valued at US$ 42.5 million) to 265

metric tons (valued at US$32.5 million) due to the presence of aflatoxins (Surendranatha *et al.*, 2011).

Aflatoxin contamination can result in direct economic impact through export rejections from importers with stringent aflatoxin regulations such as the European Union (EU) countries. Between 2007 and 2012, the EU alone has issued 346 notifications to African countries (PACA, 2012).

Aflatoxins losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency. Other adverse economic effects of aflatoxins include lower yield for food and fiber crops (Anon, 1989).

Aflatoxin contamination of peanut affects the quantity and quality produced and marketed. The aflatoxin contaminated peanut is tainted and cannot be marketed and must be thrown away. Awuah *et al.* (2009) stated that about 5 to 15 percent of peanut in Ghana were discarded during sorting. This reduces the supply of peanut marketed at the farm level. Lower quality peanut is less attractive to buyers who offer a lower price for aflatoxin contaminated peanut. Hence it is expected that aflatoxin will lower farmer revenue and increase production and marketing risks (Cynthia, 2009).

FAO estimates that many basic foods could be contaminated with mycotoxin producing fungi, contributing to huge global losses of foodstuff, about 1000 million metric tons each year (Bhat *et al.*, 2010).

2.2.2.6. Legislation

There is currently harmonized European Commission (EC) legislation on mycotoxins. In England this legislation is implemented by the Contaminants in Food (England) Regulations 2003 [S.I. 2003 No. 1478], and sets limits for aflatoxins in food including peanuts, edible nuts and their products. Parallel legislation exists for Scotland, Wales and Northern Ireland. The regulatory limits are 2 μg/kg for B1 and 4 μg/kg for total aflatoxins (Judy, 1998).

On the other hand, to protect consumers and farm animals, regulatory limits have been adopted. National and international institutions and organizations such as the EC, the US Food and Drug Administration (FDA), WHO and FAO have recognized the potential health risks to animals and humans posed by consuming aflatoxin-contaminated food and feed .In an attempt to harmonize the current tolerances to aflatoxin which exist in different countries, the working group on mycotoxins of the WHO and FAO proposed maximum limits of 15μg/Kg for total aflatoxins in raw groundnuts based on a sample size of 20 Kg (Bhat *et al.*, 1996).

The potential economic problems associated with a level of 10μg/Kg and the public health implications of a level of 15μg/Kg, as compared to 10μg/Kg for aflatoxins in foods, are two main issues in the setting of maximum levels for aflatoxins in groundnuts intended for further processing. Many countries considered the level of 15μg/Kg to be a reasonable limit that could be achieved by producing countries, thus facilitating international trade, considering that a lower level would constitute a trade barrier due to the finding of the evaluation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that this may not offer a significant improvement in public health. However, genotoxic properties of aflatoxin, uncertainties in risk assessment, the As Low As is Reasonable Achievable principle, and inadequate data on the effect of a level of 10μg/Kg on the availability of groundnuts in the world market support the continued consideration of the lower level (Anna, 2012).

Codex Alimentarius Commission and the JECFA have adopted the maximum limit for total aflatoxins as 15 μg/Kg in raw peanut, and 10 μg/Kg in peanut processed for human consumption, which means that half of those concentrations are applicable in terms of AFB1 (Codex, 2001; IARC, 1995).

The issue of food safety in Africa is one which interacts with and is frequently subject to issues of food security, especially in geographic areas where food shortages are caused by recurrent natural weather phenomena such as drought. In addition, many subsistence farming communities in Africa are reliant on the consumption of home-grown crops, irrespective of the quality considerations normally applied in the developed world. Nevertheless, some African governments have instituted food safety regulations to control mycotoxin, especially aflatoxin,

contamination of the national food supply and research into natural occurrence of aflatoxins in a range of local foods is widely conducted (RASFF, 2011)

According to the FAO (2004) at least 100 countries have specific regulations to deal with mycotoxins or with at least regulatory limits on aflatoxins; 13 countries do not have specific regulations while 50 countries, of which 40 are from Africa, have no regulations/no data exist. Only eight African countries have existing regulations for aflatoxin, viz. Cote d'Ivoire, Egypt, Kenya, Malawi, Nigeria, Senegal, South Africa and Zimbabwe.

Limits for aflatoxin in food products in Kenya are 10 mg/kg total aflatoxin and 5 mg/kg aflatoxin B1 (AFB1) in peanuts and other food grains (KE BS, 2007). However, the high cost of testing discourages the majority of the farmers, processors and traders from testing their products. This setback, in the absence of adequate knowledge, is further compounded by the reluctance of consumers to pay the extra cost of a tested product, when there is a readily available alternative (Mutegi *et al*, 2013).

East Africa countries had jointly set a limit on aflatoxin in peanut butter upto maximum of 15μg/kg for total aflatoxin and 5μg/kg for AFB1. Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community (EAC, 2013).

2.2.2.7. HPLC System

For aflatoxins analysis several methods have been developed over the last 30 years. Because of the advances in technology, the better clean-up procedures and the combination of both, a higher sensitivity has been registered, High Performance Liquid Chromatography (HPLC) with fluorescence detection becoming the most used analytical methodology in laboratory. Other advances have regarded the environment, as the replacing of chlorinated solvents, preferred in the past, by aqueous mixture of methanol or acetonitrile, that are also more compatible with antibodies, recently introduced in many applications . These reagents marked a turning point in the sample preparation step as well as in the identification phase, showing a high flexibility in many practical situations in which reliable, rapid and simple analyses are required to reduce costs (Anna, 2011).

2.3. Pathogenic point of view

2.3.1. Description on *Salmonella*

Salmonella spp. is bacteria that cause salmonellosis, a common form of food borne illness in humans. Outcomes from exposure to *Salmonella* spp. can range from mild symptoms to severe disease and can be fatal. *Salmonella* spp. is carried by a range of domestic and wild animals and birds and has been widely isolated from the environment. *Salmonella* spp. is gram-negative, non-spore forming rod-shaped bacteria and is members of the family *Enterobacteriaceae. Salmonella spp.* is classed as facultative anaerobic organisms as they do not require oxygen for growth (Jay *et al.* 2003). The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori*. Over 99% of human Salmonella spp. infections are caused by *S. enterica subsp. enterica* (Bell, 2002; Crum-Cianflone 2008).

Salmonella spp. has relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. *Salmonella spp* is influenced by a number of factors such as temperature, pH, water activity and the presence of preservatives. The temperature range for growth of *Salmonella spp.* is 5.2–46.2°C, with the optimal temperature being 35–43°C. *Salmonella* spp. will grow in a broad pH range of 3.8–9.5, with an optimum pH range for growth of 7–7.5 (ICMSF, 1996).

There is a common misconception that low numbers of *Salmonella spp* are not a problem in low moisture foods like Peanut butter because these products do not support *Salmonella* growth. However, low numbers of *Salmonella* in foods can cause illness, and the presence of the organism in low moisture ready-to-eat foods must be prevented (Neil, 2009).

Heat resistance of *Salmonella* spp. in food is dependent on the composition, pH and water activity of the food. The heat resistance of *Salmonella* spp. increases as the water activity of the food decreases. Water activity (a_w) has a significant effect on the growth of *Salmonella* spp., with the optimum a_w being 0.99 and the lower limit for growth being 0.93 (ICMSF 1996; Podolak *et al.* 2010). Foods which are high in fat and low in moisture, such as chocolate and peanut butter,

may have a protective effect against heat. In low pH conditions the heat resistance of *Salmonella* spp. is reduced (Jay *et al.*, 2003; Shachar *et al.*, 2006; Podolak *et al.*, 2010).

Spreading of contaminants can occur when nuts are exposed to untreated, recirculated water. There are likely multiple sources of *Salmonella* and *E. coli* in tree nuts and ground nut harvesting results in significant mixing of the nuts with soil and plant debris. Contaminants picked up at harvest may then be spread to the edible kernels before or during shelling. Moisture may also play a role in amplification of contaminants if the harvested and dry product is not protected from irrigation water or rainfall (Neil, 2009).

Similarly, water introduced into shelling or further processing facilities has been associated with multiplication and potential persistence of some strains within the post-harvest environment. Facility contamination may lead to sporadic contamination of finished raw and processed product. For this reason, a robust environmental monitoring program for *Salmonella* should be implemented for any facility processing, handling and/or packaging the final nut product (Empres, 2012).

The post-process contamination of peanut butter and spreads with *Salmonella* may result in survival in these products during their shelf life at 5 °C and possibly at 21 °C, depending on the formulation (Burnett *et al.*, 2000).

Although *Salmonella* cells do not multiply because of the low a_w, the organism may survive for relatively long periods of time in the product. The author concluded that reduction of *Salmonella* during storage cannot be predicted solely on the basis of a_w. Interactions between low a_w and environmental factors such as storage in air or under vacuum and temperature appear to play an important role in *Salmonella* survival. It was reported that *Salmonella* may survive for months in foods such as chocolate, peanut butter and pepper. The combination of high fat and low a_w might have a synergistic effect on *Salmonella* survival (Podolak, 2010)

Salmonella can persist for prolonged periods of time in the dry state and in low-moisture products. The ability of the organism to survive under dry and other adverse environmental conditions makes it difficult to control. Although some reduction of numbers occurs in low

moisture foods during storage, the degree of reduction depends on many factors such as storage temperature and product formulation. In challenge studies, *Salmonella* was detected in chocolate products after 1-9 month storage at room temperature, in peanut butter products after 6-month storage at room temperature and after storage for more than 6 months at refrigeration temperature (Burnett *et al.*, 2000).

In addition, study from Mali indicated that peanuts butters sold in Mali are generally prepared in a traditional way and under conditions of unhealthy hygiene. The conditions of production and storage of these peanut butters and especially their exposure to the air at the time of sale and packaging used are potential sources of contamination by pathogenic microorganisms. These contaminants can cause food poisoning and selling of poor-quality peanut butters in the markets (Babana *et al.*, 2010). In addition, the consumption of more and more of peanut butters expose more people to serious diseases (typhoid fever, diarrhea). So it is urgent to act, and quickly, to deal with such an eventuality (Keita *et al.*, 2013).

2.3.2. Symptoms of disease

Outcomes of exposure to non-typhoidal *Salmonella* spp. can range from having no effect, to colonizing of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonization with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhea, mild fever, vomiting, dehydration, headache and/or prostration (WHO/FAO, 2002).

Severe disease such as septicaemia sometimes develops, predominantly in immune compromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as arthritis, appendicitis, meningitis or pneumonia as a consequence of infection (Hohmann, 2001; WHO/FAO, 2002; FDA, 2012).

Salmonellosis is one of the most commonly reported enteric illnesses worldwide, being the second most frequently reported cause of enteric illness in Australia (behind campylobacteriosis) (Jay *et al.*, 2003).

2.3.3. Incidence of illness and outbreak data

Outbreaks attributed to *Salmonella* spp. have predominantly been associated with animal products such as eggs, poultry, raw meat, milk and dairy products, but also include fresh produce, salad dressing, fruit juice, peanut butter and chocolate (Jay *et al.*, 2003).

Salmonella outbreaks from low-moisture products are relatively rare but often impact large numbers of people. In the US between 1996 and 2006, of 64 outbreaks (with 5981 cases) of salmonellosis reported for FDA-regulated foods (excluding eggs), only 2 were from low-moisture processed food products. However, the two outbreaks attributed to low-moisture food products (toasted oats cereal and peanut butter) involved a large number of illnesses. During the course of the outbreak investigations, CDC reported 628 cases attributed to peanut butter in 47 states between August 2006 and May 2007. These two outbreaks eventually accounted for 1037 clinically confirmed cases of illness (CDC, 2007).

Moreover, a second major *Salmonella* outbreak in the US attributed to peanut butter and products containing peanut-derived ingredients involved more than 500 cases in 43 states between September 2008 and January 2009. Overall, more than 700 infections and 9 deaths were reported as of April 20, 2009, and resulted in more than3, 900 food products being recalled, resulting in the largest food recall in U.S. history (Neilh, 2009; PEM, 2009; Lindhardt, 2009).

A survey of food-borne outbreaks in the United Kingdom found cross-contamination to be a significant contributing factor in 32.1% of the cases (Powell, 1998). An investigation of *Salmonella* spp. Cross-contamination in an oilmeal processing plant found that controlling traffic flow (personnel and materials), rodents and airborne dust were key factors in reducing contamination rates (Morita *et al.*, 2006). In two major peanut butter salmonellosis outbreaks, uncontrolled dust in the production facilities was thought to be a major contributing factor (Almond, 2009).

Survey conducted by the World Health Organization (WHO, 1995) that indicated a significant proportion of European food borne outbreaks could be traced back to cross contamination. The report indicated that factors contributing to the presence of pathogens in prepared foods included insufficient hygiene (1.6%), cross contamination (3.6%), processing or storage in inadequate rooms (4.2%), contaminated equipment (5.7%), and contamination by personnel (9.2%). In a compilation of outbreaks in the United Kingdom where the contributing factor was known, cross contamination accounted for 57% of all occurrences (Powell *et al.*, 1998).

Most outbreaks underscore the difficulty in eradicating *Salmonella* from the environment of dry product manufacturing facilities and illustrate the wide diversity of low-moisture products that can be contaminated with *Salmonella* and cause illness. These outbreaks also highlight the need to reinforce industry preventive control measures through guidance based on the best available information. Because of the outbreak of food borne illness linked to *Salmonella* contamination of peanut butter, AOAC International's subsidiary Research Institute, Gaithersburg, Md., launched a new program, Emergency Response Validation, designed to respond immediately to emerging food contamination crises by rapidly evaluating test kits for specific bacteria in specific foods once a crisis is identified (Neil, 2009).

The presence of *Salmonella* in low-moisture products is a concern because low numbers of *Salmonella* in foods can cause illness. This is contrary to a common misconception that low numbers of *Salmonella* are not a problem in low-moisture foods because these products do not support *Salmonella* growth. *Salmonella* does not need to grow to cause illness; in some instances infection has occurred from consuming low-moisture products contaminated with less than 1 cfu/g depending on the host, the product, and the *Salmonella* strain. For example, several incidents involving low numbers of *Salmonella* in chocolate have been reported over the years. In an outbreak attributed to paprika and paprika-powdered potato chips, *Salmonella* was found at 0.04-0.05 cfu/g in the snacks. In the 2006-2007 outbreak associated with peanut butter, *Salmonella* was found at 1.5 MPN/g in an unopened jar and a lower level was found in another product sample (Yuhuan, 2009).

2.3.4. *Salmonella* control elements

The key factors include the control of employee traffic patterns to minimize the possibility of cross-contamination and the control of rodents and wild birds. The International Commission on Microbiological specification for Foods (ICMSF) recognizes that, while it is not possible to prevent the introduction of pathogens into food processing facilities, it is crucial to minimize their presence (ICMSF, 2002).

Contamination of low-moisture products with *Salmonella* is of concern in operations without an inactivation step (such as a dry-blending operation) or when it occurs after the inactivation step. *Salmonella* outbreaks associated with low-moisture products may occur due to the inclusion of contaminated (raw) ingredients, insufficient processing, or post-processing contamination (Codex, 2008).

According to Codex (2008), to minimize the risk of *Salmonella* contamination the following seven elements should be applied to control *Salmonella* in low-moisture products:

1. Prevent ingress or spread of *Salmonella* in the processing facility.
2. Enhance the stringency of hygiene practices and controls in the Primary *Salmonella* Control area.
3. Apply hygienic design principles to building and equipment design.
4. Prevent or minimize growth of *Salmonella* within the facility.
5. Establish a raw materials/ingredients control program.
6. Validate control measures to inactivate *Salmonella*.
7. Establish procedures for verification of *Salmonella* controls and corrective actions.

All of these issues are relevant to the production of peanut butter so as to minimize contamination of pathogens like salmonella.

According to joint statement by the WHO-WFP-UNSCN-UNICEF (2007), the tolerance limit of *Salmonella* in ready to use therapeutic foods should be negatice or zero per 125g of test sample.

2.4. Control of contaminations as a general

As a control of any contamination from products, different measure could be applied. As is implied in the expressed hope that "with further improvements in agricultural practice it would become feasible to insist on lower levels" the best approach is prevention. And the first step is recognition and awareness that the problem and threat exist. A major problem is motivation of untrained personnel at all stages of culture, harvest, transportation, and processing. Ccontaminations in agricultural products may occur, while plants are growing in the field, during harvesting and handling, during storage, and even during processing. According to different authors, high moisture is the single most important condition contributing to mould. Temperature is also very important, but moisture control is crucial in mould and bacterial prevention. The safe moisture level varies not only with the crop, but also with the length of time it is to be left in storage, the temperature of storage, weather conditions in the area, the design of the storage facilities and still other factors. (Leo, accessed on October 25, 2013).

Dohlman (2003) proposed a strategy to reduce both health risks and the economic costs associated with mycotoxins. Food producers must be more aware of mycotoxin effect, and therefore, handling practices that would minimize mycotoxin contamination. Moreover, another solution would be to encourage the adoption of process-based guidelines (good agricultural practices or good manufacturing practices (GMPs).

Several cultural practices such as sorting, sieving, tumbling, winnowing and drying, that were used by the traders are recommended as ways of reducing aflatoxin contamination of peanuts. Sorting out physically damaged and infected grains from produce can result in 40 to 80% reduction in aflatoxin levels (Mutegi *et al*, 2013).

In addition, based on Eshetu's (2011) recommendation, electronic sorting and hand-picking of damaged, immature or mould-infested pods can significantly reduce the contamination of groundnuts in shell. Good hygienic practice is the way to prevent salmonella contaminations.

Botwana government had banned peanut butter imported from South Africa because of higher aflatoxin content of the product (Republic of Botswana press release, 2012). However, no agreable information was obtained regarding control of such items in our case, Ethiopia.

3. Methods and Materials

3.1. Description of the study site:

The study was conducted in Addis Ababa. Addis Ababa has 10 sub cities having woredas in each of the sub cities. Addis Ababa is a major trade center for peanut with many cottage industries that process raw peanut, roasted and splitted peanut, and peanut butter. In addition, the addresses written on packaging of peanut butter products indicate the site of their establishement; which supports the evidence that most processers of peanut are found in Addis Ababa. This clearly indicates that there was a high demand of peanut and peanut products in Addis Ababa, hence, it was chosen as a study site.

Samples were collected from all margins of the city and analyzed at Bless Agri Foods Laboratory (accredited laboratory in ISO 17025 requirements for nearly 20 chemical and microbial parameters; sister company of Hilina Enriched Foods PLC). The laboratory is established at the heart of Legetafo town, 20km far away from Addis Ababa to the North East.

3.2. Sampling technique

Samples of peanut butter were selected from a variety of retail outlets, including supermarkets, smaller shops and market stalls across Addis Ababa. A wide range of brands was covered in order to ensure that the survey was representative of the supply of the products to consumers in Addis Ababa. Before sampling was employed, sampling sites were divided in to four direction for ease of sampling; North, South, East, West, so as to address most locations and minimize missed brands. All brands of peanut butter obtained during sampling periods were included in the study. Of course, the absence of a particular brand means only that the product was not included in this survey. So, out of 48 samples, 40 samples were from different brands and the remaining 8 samples were selected twice during course of sampling. For roasted peanut, samples were collected from Merkato (potential area) and different streets of Addis Ababa based on the availability.

3.3. Sample collection

A total of forty eight samples of peanut butter were randomly selected from different supermarkets and retailers for both Aflatoxin and *Salmonella* analyses. All kind of brands were collected from North, South, East and West to simply address all sampling points. Accordingly, sample distribution scenario was; 12 samples (9 were different brands) from North; 11 samples (10 were different brands) from South; 13 different brands of samples from East and 12 samples (8 were different brands) from Western part of Addis Ababa. Out of these 48 samples, 45 samples were from local producers and 3 samples were imported products (collected from East, West and South).

Sample collection sequence was East, West, South and then North.

In addition, a total of ten samples of roasted peanut each weighing 3 kg were randomly collected from street vendors and distribution point (Merkato).

Approximately 1 kg of each type of peanut butter brand was taken. Where the product was sold in packs of less than 1 kg, a number of retail packs were purchased, ensuring that all came from the same batch, and these were mixed thoroughly before taking a sample for analysis.

3.4. Laboratory Analyses

3.4.1. Procedure for Aflatoxin analyses

3.4.1.1. Apparatus

HPLC system consisting of auto sampler, pump, column oven, fluorescence detector, PC with chromatography software, Photochemical reacter for post column derivatization system, HPLC column, Weighing balance, Immunoaffinity column, AflaCLEAN™ (LCTech P/N 10514), refrigerator, spatula, Beakers (25ml, 100ml, 250ml & 500ml), funnel, high-speed blender, Single-use syringes (10 mL (PP), 0.2 µm filter disks, sample preparation vials with screw cap and sealing ring, ppipettes (1ml & 10ml), whatman filter paper, Vaccum pump, lab tissues and other necessary laboratory glass wares and equipment were used for the analyses of the aflatoxin at the Bless Agrifood laboratory.

3.4.1.2. Chemicals and reagents for aflatoxin analysis

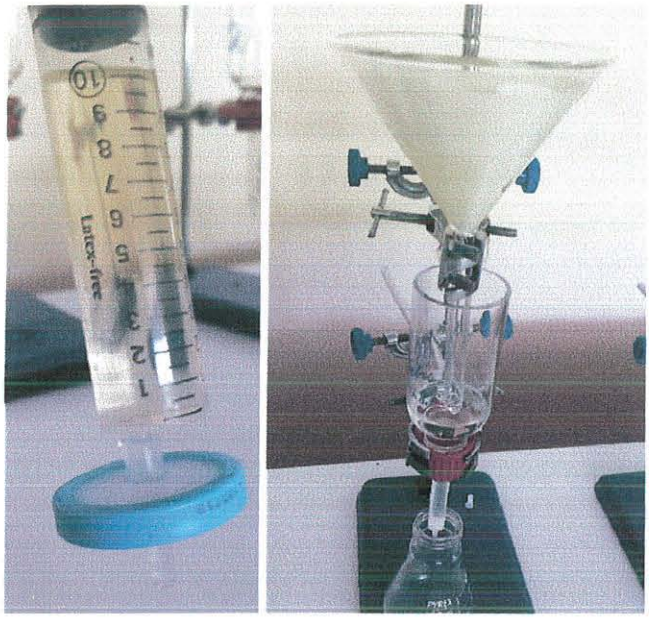
Chemicals and reagents used for Aflatoxin analyses were: HPLC grade water, acetonitrile, Na_2HPO_4 * $12H_2O$, n-Hexene, NaCl, methanol, aflatoxin standard. The AFB1, AFB2, AFG1, and AFG2 standard were obtained from Sigma- Aldrich (Ref A6636 – Batch No. 118K4094). The pure reference standards were stored in the dark at 4°C. Standard solutions of all four aflatoxins were prepared in methanol and stored in the dark at 4°C for up to 6 months.

3.4.1.3. Sample extraction

A well mixed composite 20g of sample and 2g of sodium chloride was weighed and put into high-speed blender jar. An extraction solutions; HPLC grade methano/ HPLC grade water (80:20, v:v) and 50ml of n-hexane were measured and added into the jar and blended for 3 minute at high speed. The homogenized sample was then filtered through a plaited filter paper The oily part was again separated from the aqueous layer by suspending for some minutes into funnel. The oily part was suspended on the upper part of aqueous layer . The aqueous layer was collected at the bottom of the funnel. Extractions of aflatoxin from the test samples were performed according to the official methods of AOAC 2005.08.

3.4.1.4. Clean up

Accurate 7ml of the aqueous sample filterate was taken by glass syring barrel in which its tips was fitted with 0.2 μm filter disks for better filteration. A 43ml of PBS-buffer (PH 7.2) solution and the measured 7ml sample were mixed together and passed through the prepared immune affinity column (Before adding the mixture, the immune affinity column was openned to drain the storage buffer until the level reached the upper frit) at a flow rate of 2-3 ml/min. The sample let to drain through the column until there was no more sample in the column and then the column was washed with 10 ml distilled water and residual water was expelled from the immuno affinity column by a gentle gas stream or vacuum. The retained aflatoxin in the column was eluted by 2ml of HPLC-grade methanol. All of the methanol elution was collected into the sample vial and ready for injection into HPLC system. The preparation procedure was employed based on the method described by AOAC Official Method 2005.08(24).



A B

Figure 2: A/ Glass syring barrel in which its tip was fitted with 0.2 µm filter disk; B/ Immune affinity column fitted with filter paper.

3.4.1.5. Executing analysis of aflatoxins

HPLC Conditions:

Column:	Zorbax Eclipse plus C18, 4.6 x 150 mm x 5 µm
Column Temp.:	39 °C
Mobile Phase A:	HPLC grade water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile
Isocratic:	A : B: C; 65: 25: 15, 12min
Flow rate:	1.0 mL/min
Detection:	Ex: 355 nm, Em: 455 nm, gain = 10, attenuation=4, fluorescence.
Injection:	20 µL

Electrochemical Current: 100 µA setting

Reaction coil: 0.5 mm i.d.*34 cm long peek tubing (From the exit of KOBRA cell to the entrance of FLD)

The limit of detections of aflatoxin G1 and B1 were improved by post-column electrochemical bromo-derivatization and fluorescence detector. The analysis was shortened into 8 minutes without compromise of resolution of aflatoxins (in order to completely elute all the component in sample, the analysis time was set for 11 minutes). Meanwhile, it was shown from the chromatograms for real samples that the cleanup of sample was effective by pre-column immune affinity column. It was proved that the method in the work could be applied for determination of aflatoxins at trace level in real samples, and method is fast and accurate.

Derivitization

Since only the aflatoxins B2 and G2 readily fluorescence, aflatoxin B1 and G1 must be derivatized prior to detection. Accordingly, this was achieved photo chemically by irradiation with UV light at 254nm with a photochemical reactor (LC Tech UVE, Germany).

Fig.3: The photochemical reactor UVE™ for post-column derivatization

HPLC determination

The eluted aflatoxin-methanol solution was directly measured by HPLC for its aflatoxin content using HPLC conditions as described in the method.

3.4.1.6. Analytical Quality Assurance

3.4.1.6.1. Materials

Pure reference standards for the different aflatoxins were obtained from Sigma-Aldrich and included aflatoxin B1 (Ref A6636 – Batch No. 118K4094), aflatoxin B2 (Ref A9887 – Batch No. 029K4062), aflatoxin G1 (Ref A0138 – Batch No. 069K4001) and aflatoxin G2 (Ref A0263 – Batch No. 018K4019). The pure reference standards were stored in the dark at 4°C.

Standard solutions of all four aflatoxins were prepared in methanol and stored in the dark at 4°C for up to 6 months.

Standard reference material (SRM) of aflatoxins in peanut butter was obtained from NIST (Standard Reference Material® 2387) and stored in the dark at -20°C.

Maize proficiency test material: was obtained from FAPAS (FAPAS® Proficiency test 04166).

The validation was performed using replicate analyses of the standard solutions, standard reference material and proficiency test samples mentioned above.

3.4.1.6.2. Linearity

A series of standard curves were prepared over a concentration range of 5 – 50 µg/l (5, 10, 20, 30, 50.0 µg/L) from a stock solution of total aflatoxins in methanol. The concentration of the individual aflatoxins ranges from 0.5 to 5 µg/L for AFG2; from 2 to 20 µg/L for AFG1; from 0.5 to 5 µg/L for AFB2 and from 2 to 20 µg/L for AFB1. Dilutions were prepared in the mobile phase. Each solution was injected as HPLC conditions described above and the data obtained from peak area versus each aflatoxin was treated by linear least square regression analysis. The correlation coefficients (R^2) were calculated for a standard solution and the minimum linearity was (R^2) $\geq$ 0.9972) (Appendix B). The standard curves were evaluated for reproducibility.

Table 1: Calibration curves for aflatoxins

Aflatoxins	Range	Linear regression analyses (y=bx+a)		
		a	b	Correlation coefficient (R^2)
AFG2	0.5-5µg/kg	0	0.07108	0.9980
AFG1	2-20µg/kg	0	0.02578	0.9972
AFB2	0.5-5µg/kg	0	0.13560	0.9990
AFB1	2-20µg/kg	0	0.05231	0.9990

Quantification of the actual aflatoxin in ppb (µg/L or µg/kg) is based on the following formula (AOAC 2005.08):

Aflatoxin, ng/g (ppb) = A x (T/I) x (1/W)

Where:

A = ng of aflatoxin as eluated

T = final test solution eluate, volume (µL/)

I = volume eluate injected into Lc (µL)

W = mass (g) of commodity represented by final extract

3.4.1.6.3. Detection limit

The lowest concentration in a sample that can be detected, but not necessarily quantified, under test condition were determined by the aflatoxin concentration yielding a signal-to noise ratio of at least 3:1(S/N $\geq$ 3). The limit of detection was measured for all 4 aflatoxins by injecting standard solutions of different concentrations and measuring for each substance the amount injected on the column and the S/N values.

The signal-to noise ratio was determined by the equation:

S/N = 2H/h

Where:

H: height of the peak corresponding to the component

h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

Approximate LOD values are summarized in the table below and range from 3 to 13 pg.

Table 2: Limit of detection (LOD) of aflatoxins

Aflatoxins	ng injected on column	S/N	LOD, pg
AFG2	0.010	6.3	4.7
AFG1	0.040	9.0	13.3
AFB2	0.010	9.3	3.2
AFB1	0.040	15.3	7.8

3.4.1.6.4. Accuracy and Precision

The accuracy and precision of the method were determined by single or replicate analysis of samples containing known aflatoxin amounts. For that purpose, test standards (prepared separately from the calibration standards), the standard reference material (SRM) and the proficiency test sample were used.

The results for the test samples are given below. The accuracy ranged from 94.2 to 108.3 %

Table 3: Accuracy of the method for the determination of aflatoxins in test samples

Test sample	Aflatoxin	ug/L added	ug/L found	Accuracy
1	AFG2	1.00	0.94	94.4
	AFG1	4.00	3.77	94.2
	AFB2	1.00	1.08	107.5
	AFB1	4.00	4.17	104.3
2	AFG2	2.00	2.07	103.3
	AFG1	8.00	8.29	103.7
	AFB2	2.00	2.14	106.9
	AFB1	8.00	8.66	108.3

The duplicate results for the SRM sample are given below. In these analyses, the crude peanut butter extract was analyzed in duplicate using two different Immunoaffinity columns. The accuracy ranged from 96 to 111 % and the precision from 3.6 to 3.7%.

Table 4: Accuracy and precision of the method for the determination of aflatoxins in SRM (peanut butter) samples.

Aflatoxin	ref. conc.	ug/kg found (1)	ug/kg found (1)	mean	S.D.	C.V.	Accuracy
AFB2	0.70	0.76	0.80	0.78	0.03	3.6%	111.4%
AFB1	4.20	3.92	4.13	4.03	0.15	3.7%	95.8%

Results for the replicate analysis of the proficiency test sample in maize are given below. The accuracy and precision (C.V.) ranged from 88.3 % to 99.0 % and from 2.1 to 9.4%, respectively.

Table 5: Accuracy and precision of the method of aflatoxin in a proficiency test sample (maize).

		ug/kg found										
Aflatoxin	ug/kg added	1	2	3	4	5	6	7	mean	S.D.	C.V.	Accuracy
AFG2	2.2	1.94	1.57	1.71	1.77	1.97	2.02	2.02	1.86	0.18	9.4%	95.7%
AFG1	2.28	2.61	2.13	2.04	2.39	2.36	2.25	2.36	2.31	0.19	8.1%	88.3%
AFB2	2.09	2.31	2.15	2.2	2.25	2.19	2.23	2.26	2.23	0.05	2.4%	96.4%
AFB1	6.53	6.67	6.64	6.39	6.75	6.45	6.72	6.59	6.60	0.14	2.1%	99.0%
Total AF	12.72	13.53	12.49	12.34	13.16	12.97	13.22	13.23	12.99	0.43	3.3%	96.0%

3.4.1.6.5. Over all repeatability

The reproducibility of the method was determined by replicate extraction (2 samples), cleanup analyses (2 replicates per extract) and injections (3) of the maize proficiency test sample. The between-assay reproducibility, expressed as the coefficient of variation (C.V.) ranged from 1.5 to 20.8 % for the different aflatoxins and was 3.8 % for the total sum of aflatoxins. Looking at the within-assay reproducibility, it appears that there is an excellent precision for the different injections, but a somewhat lower reproducibility (higher C.V. values) for the clean-up analyses. The results for all four aflatoxins were summarized in the table below.

Table 6:_Reproducibility of the method for the determination of aflatoxins in maize.

Aflatoxin	Within-assay (Injections) n=3	Within-assay (clean-up), n= 2	Between-assay (overall method) n=2
AFG2	3.4%	18.4%	20.8%
AFG1	4.0%	31.0%	18.2%
AFB2	1.3%	4.7%	1.5%
AFB1	2.5%	6.2%	3.3%
Total AF	2.1%	2.4%	3.8%

3.4.1.6.6. Recovery

The recovery of standards was processed with every batch of sample. The sample was used to assess recovery with value between 60 and 120% classed as valid (RASFF, 2011). Accordingly, The extraction recovery of AFB1 and AFB2 from the Immunoaffinity columns was measured by spiking 40-ml volumes of crude extracts of standard reference material (assigned value 4.13 µg/kg AFB1 and 0.80 ug/kg AFB2) with additional amounts of aflatoxins (10 ng AFB2 and 40 ng AFB1 in 5ml of methanol) corresponding to total theoretical concentrations of 5.68 ug/L AFB1 and 1.28 ug/L AFB2. These spiked samples were then loaded onto the immunoaffinity column and analyzed in duplicate by the HPLC method. The results are tabulated below.

Table 7: Extraction recovery of AFB1 and AFB2 from peanut butter.
(SRM sample spiked with additional amounts of aflatoxins)

Aflatoxins	Theoretical conc., µg/kg	Trial 1		Trial 2	
		Concentration found, µg/kg	Recovery	Concentration found, µg/kg	Recovery
AFB1	5.68	6.58	116%	6.50	114%
AFB2	1.28	1.05	82%	1.09	86%

% Recovery = (Result found/ Result expected) X 100

3.4.1.6.7. Validation of the Method

Bless Agri Food Laboratory Service PLC participated in a proficiency test to assess the inter-laboratory accuracy and precision by analyzing a proficiency test sample in maize. The results are summarized in the table below and demonstrated the validity of the method.

Table 8: Results of the proficiency test for aflatoxins in maize.

Aflatoxins	Value assigned, µg/kg	Value found, µg/kg	Analytical recovery	Z-score
AFB1	6.53	6.60	101.1	0
AFB2	2.09	2.23	106.7	0.3
AFG1	2.28	2.31	101.3	1.1
AFG2	2.20	1.86	84.5	-0.7
Total AF	12.72	12.99	102.1	0.1

3.4.2. Procedure for *Salmonella* analyses

Samples collected from Addis Ababa Supermarkets for *Salmonella* analyses was tested at Bless Agri Food Laboratory Service PLC based on the AOAC 967.25.

3.4.2.1. Equipment and Materials

Blender (stomacher), sterile Erlenmeyer flasks of various volumes, mass balance, incubator (35 ^{0}C ±2 ^{0}C), water bath 49 ^{0}C ±1 ^{0}C, sterile spoons for sample transfer, sterile culture dishes (15 x 100 mm), glass or plastic, sterile pipettes (with 0.01 ml graduations, 5ml and 10ml witht 0.1 ml graduations), inoculating needle and inoculating loop, sterile test or culture tubes, PH meter and other miscelaneous materials.

Reagents: KI/I solutions, Brilliant green solution (1%), Alpha-naphthanol, Metyl red solution, Kovac's.

3.4.2.2. Media and Reagents

Lactose broth,selenite cystine (SC) broth, Tetrathionate (TT) broth, Xylose lysine desoxycholate (XLD) agar, Hektoen enteric (HE)agar, Triple Sugar Iron Agar, Lysin Iron Agar, Bismuth sulfite (BS),MR-VP broth, simmons citrate agar, MacConkey agar,. Positive control was *Salmonella enteritidis* and blank run was an inoculated nutrient media.

3.4.2.3. Sample preparation

3.4.2.3.1. Pre enrichemenet media:

Lactose broth was used for dilution purpose. Representative and homogenized samples of 25g was weighed in sterilized Erlenmeyer flask (250ml volume). The test sample was incubated with previously sterilized lactose broth for 24hr ± 2hr at 35^{0}C ± 1^{0}C to favor the repair and growth of stressed or sub lethally injured *Salmonella* arising from exposure to hear, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations.

3.4.2.4. Sample analyses

3.4.2.4.1. Selective enrichement

Media used wee Tetrathionate broth and Selenite Cystine broth: After incubating the sample for 24hr ± 2hr at 35^0C ± 1^0C appropriate pre-enrichment medium, proceeded by transferring 1ml sample homogenate on to Tetrathionate (TT) and Selenite cysteine (SC) broths. Incubate TT and SC broth at 35^0C ± 1^0C for 24hr ± 2hr. The analyses were conducted in duplicate in each step.

3.4.2.4.2. Selective plating

Enrichment cultures were streaked onto selective differential agars. Hektoen Enteric agar, Bismuth sulfite (BS) and Xylose lysine desoxycholate (XLD) agar for the isolation of *Salmonella*. Each agar was incubated for 24hr ± 2hr at 35 ^{0}C.

According to typical colony characteristics depicted in each agar, detection of *Salmonella* was checked. Blue-green to blue colonies with or without black centers was verified in HE agar. Pink colonies with or without black centers in XLD; brown, gray or black or methalic sheen in BS were veried for presence of *Salmonella*.

If no typical colony characteristics were observed, the result of *Salmonella* in the test sample was reported as absent or not detected. In case of any observation of typical colony characteristics, further confirmatory test was performed (as indicated in screening test).

3.4.2.4.3. Screening test

Triple Sugar Iron Agar (TSI), Lysin Iron (LI) Agar, XLD Agar, Hektoen Enteric Agar, MacConkey agars were used in the screening test. The medium that showed typical colony characteristics was inoculated in TSI and LI for 24hr ± 2hr at 35 ^{0}C. The presence of alkaline slant and acidic (yellow) butt with or without blackening was checked in TSI agar. Purple slant or purple butt was also checked in LI. In the absence of typical or suspicious *Salmonella* colonies, isolation step and biochemical confirmatory test were conducted. Media used in isolation step were: HE, XLD and MacConkey Agars. Typical colony characteristics in isolation step were similarily checked for HE and XLD as indicated in selective plating and colorless / transparent colony was checked for MacConkey agar. Media used in biochemical confirmatory

test were: MR-VP broth, tryptophane broth and Simon's citrate agar. MR-VP and Simon's citrate were incubated for 96hrs ± 2hrs at 35^{0}C. Tryptophan broth was checked after 24hrs of incubation period at 35 ^{0}C. Typical colony characteristics in biochemical confirmatory test were: red or pink color on the surface in MR-VP broth; lack of red color at the surface in tryptophan broth; blue slant.

3.5. Determination of oil separation

Composition of Peanut butter may vary and it may contain added palm oil or partially hydrogenated vegetable oil (EFSA, 2011). The most serious problem of natural peanut butter is the tendency of the oil to separate during storage as it has negative impact on purchase and general quality of peanut butter. The samples under this investigation were checked if there were oil separation or not. Aryana *et al.* (2000) explained what oil separation mean; it is characterized by the absence of two layers of oil and meal phase during ordinary conditions of storage. Hence based on this principle, through mere observation, the oil separation was checked (Table 14). The extent of surface oil is explained by the word "very noticeable" (borrowed from EAS document) if the surface oil is very visible and could be flow or poured.

3.6. Data analysis

Data obtained during the test of aflatoxin was analyzed and reported by mean ± *SD*, media, weighted mean and, percentage using Microsoft excel. Regression analysis was also used to determine the linearity of the results for validation. Regarding *Salmonella* analyses, the percent of the detection out of the total samples was reported.

4. Result and Discussion

4.1. Contamination level of Aflatoxin in Peanut butter

4.1.1. Local peanut butter

All 48 peanut butter samples were collected and assayed in duplicate for total aflatoxin contents. Out of these 48 samples, 45 samples were local products and the mean value for each sample was calculated. From total of 45 samples, 42 samples (93.33%) showed aflatoxin contamination by the level ranged from 3.92µg/kg to 547.85µg/kg. These samples showed a mean total aflatoxin of 98.24µg/kg ± 111.18*SD* (The median 54.56µg/kg). Great variation of aflatoxin contamination was observed (Appendix A) among samples of peanut butter as standard deviation is greater than mean value (Figure 4). About 7% (6.77) of samples (n=3) showed below detection limit.

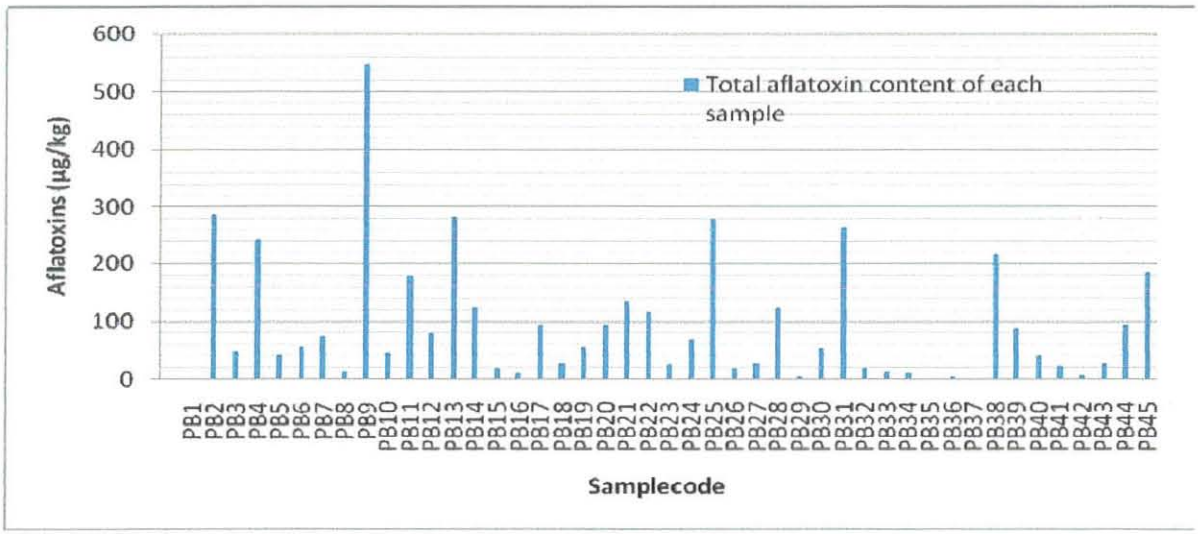


Fig. 4: Distribution of aflatoxin contamination in local peanut butter samples (see Appendix A)

Samples of peanut butter were systematically collected from supermarkets and small retailers and the incidence of aflatoxin contamination in this product was found to be 93.33%. This percentage of incidence agrees with 90% of incidence in Mali (keita *et al* 2013), but with less mean value (incomparable) of aflatoxin contamination than this study.

In contrary, the incidence of aflatoxin in this study is much higher than most findings from other countries. Report from Omer *et al* (2001) showed less percentage of contamination of the peanut butter (64%) from samples collected in Sudan. Another report of Mutegi *et al* (2013) elucidates that, 69% of peanut butter products had aflatoxin levels exceeding 10 mg/kg in Kenya. In addition, the result of present study completely differes from peanut butter of Papua New Guinea in which all samples have below < 20μg/kg with mean value 3.8μg/kg (Chilaka, 2012). In other studies, A Korean survey of different nuts and their products marketed in South Korea showed that 9 out of 85 samples including peanuts and peanut butter, were contaminated with aflatoxins (10.6% of incidence). Study by Ismail *et al* (2010) conducted in Malaysia , revealed that from about 196 nuts and their products including peanut butter, 16.3% of the products showed contamination; both studies showing incidence of aflatoxin contamination much less than this study (93.33%). The higher aflatoxin incidence in this study, comparing with others, might arise from absence of understading about quality of peanut, more specifically aflatoxin, among peanut butter processors of Ethiopia. However, the range of aflatoxin concentration of this study was 3.92μg/kg to 547.85μg/kg which is slightly comparable with that of Ismail *et al* (2010), between 17.2 to 350 μg/kg; But, compared to this study, higher aflatoxin content (841 μg/kg) was recorded in ground nut paste in Ghana during 2010 (RASFF, 2011).

Omer *et al.* (2001) reported that aflatoxin B1 causes liver cancer in Sudan. According to Yousif *et al.* (2010), aflatoxin contamination in Sudanese groundnut and groundnut products and found that percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively which is still less than contamination level of present study interms of peanut butter.

Moreover, aflatoxin result of the present study is much higher than the result reported by Mutegi *et al* (2010) which showed aflatoxin levels up to 22μg /kg in peanut butter.

According to present study, the four types of aflatoxin components were detected in the different ranges; AFB1, AFB2, AFG1 and AFG2 were detected by the concentration range of 2.65 to 270, 0.67 to 21.81 μg/kg, 0.87 to 247.1 μg/kg and 0.5 to 13.03 μg/kg, respectively.

Among the naturally occurring aflatoxins (B1, B2, G1 and G2), aflatoxin B1 is the most important for humans and animals by its frequency and toxic compound and in this study, it was

found to be the most contaminating type of aflatoxin in 93% of total samples, followed by AFG1 (88.89%). This order of contamination (AFB1 & AFG1, AFB2, AFG2) was similar with report of Yousif *et al* (2010) and Eshetu (2010), although Eshetu's was in raw peanut. They confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2 which agreed with the finding of the present study.

Occurrence of AFB1 from 45 sampled peanut butter under the present study indicated that 42 (93%) were contaminated at a level ranging from 2.65 to 270 μg/kg with mean value of 51.94 μg/kg ± 65.33 SD . This range of AFB1 contamination is in line with result of Ali *et al* (1999) conducted in Philippines ranged from 1.00 μg/ Kg to 244 μg/ Kg, but differing in percentage of contaminated samples (60%). In other way, 93% of samples in this study showed contamination by AFB1 than samples studied in philippines with 60% contamination but having comparable amount of AFB1. Samples of Peanut butter collected from western Sudan (Omer, 2001) had mean± *SD* of AFB1 much higher (87.4±197.3 μg/kg) than mean± *SD* value of present study (51.94 ±65.34 μg/kg).

Moreover, Omer (2001) reported that the AFB1 concentrations in peanut butter was between <1 μg/ /kg (detection limit is 1 μg/kg) and 781μg/kg. Sixty-three percent of the samples from West Sudan were above the permissible level of 10 μg/kg, and 50% of the samples from Central Sudan exceeded this level. Higher concentration was observed in Sudanese survey. however, 84% of samples of present study showed above 10 μg/Kg which could be showing higher contamination than study of Sudan.

Nevertheless, finding of present study is significantly much higher than that of Shami *et al* (2000) whose report indicated less contamination of AFB1 in peanut based product conducted in sudan and Botswana ranging (3.2 – 16.00 μg/ Kg).

Hence, for the justification of contamination of products, this study shares the idea of Ali *et al.* (1999), when the initial content of aflatoxin was high in the raw shelled peanut, a high level of aflatoxin contamination can be expected in its final products such as peanut candy and peanut butter. On the other hand, the low level of aflatoxin contamination in the peanut products has always been associated with the use of high quality raw materials (raw shelled peanut) that

contain an acceptable low initial level of aflatoxin. Contamination level of aflatoxin in samples of peanut butter was high. Most of the peanut butter in Addis Ababa is made by cottage scale manufacturers, from nuts that elude inspection mechanisms and are therefore likely to be contaminated. Considering that peanut butter is not a product constituting of whole kernels and that its final presentation does not exhibit physical aspects of deterioration of nuts, traders and processors are likely to take advantage of this and channel low grade peanuts for making peanut butter. Such nuts, which are cheaper than wholesome kernels, are characterized by discoloration, shriveling, and breakages, aspects that have been positively linked with aflatoxin contamination. Hence, processors may not sort out fungally infested kernes due to higher cost of peanut in the market.

4.1.2. Imported Peanut butter

From total sampled peanut butter products (n = 48), only three samples were imported product found from the market during sampling period. The reason for limited imported brands in Addis Ababa market may be due to very high price compared to local brands. Meaning, the price of one imported brand product was more than 5 times higher than the price of local brand product. As a result, very limited imported peanut butter brands were found in the market. Thus, comparison between local and imported brands of peanut butter in terms of aflatoxin contamination was not feasible.

Table 9: Aflatoxin results of imported peanut butter

	Aflatoxin (µg/kg)				
Code of sample	G2	G1	B2	B1	Total
PB46	ND	ND	ND	ND	ND
PB47	ND	ND	ND	ND	ND
PB48	ND	1.19	ND	1.09	2.28

ND = Not detected

The aflatoxin content of all imported peanut was under acceptance range of any international limit. The minimum result was ND and the maximum contamination was 2.28µg/kg.When compared to local products, only three samples (7%) from local products showed below detection limit.It indicates that imported peanut butter might be imported from country where

regulation of aflatoxin is implemented. Result of aflatoxin from local producers showed that export of the product to any country implementing regulatory limit might not possible due to higher contamination of aflatoxin. Eventhough comparison between local and imported peanut butters is not feasible due to very limited number of samples from imported products; contamination of local products by aflatoxin is higher than imported products as all imported products showed below acceptance limit (Table 9).

4.2. *Aflatoxin Results of Roasted Peanut samples*

Samples of roasted peanut were collected from different area of Addis Ababa sold by street vendors and shops so as to investigate the level of aflatoxin. Those samples would be considered as ready to eat roasted peanuts as snacks. Mostly roasted peanuts have been packed or mixed with other roasted cereals/legumes in small amount and sold in the markets. During the investigation, it was observed that most retailers of ready to eat roasted peanut collected their item from one area, even from limited shops, in Merkato. Very few of them (according to verbal communication) bought from other places. Representative 10 samples were collected from retailers and shops and analysed in duplicate for their aflatoxin content as depicted in the table below.

Table 10: Average aflatoxin results of roasted peanut samples (mean ± *SD*).

Sample code	Aflatoxins (µg/kg)				Total
	G2	G1	B2	B1	
RP1	ND	1.56±0.09	1.46±0.07	10.04±0.56	13.06±0.40
RP2	ND	5.37±0.1.65	20.1±10.02	141.79±39.90	167.59±48.39
RP3	ND	ND	1.19±0.15	10.9±0.53	12.1±0.69
RP4	1.41±0.29	3.01±0.1.29	1.2±0.57	4.13±0.1.41	9.75±0.73
RP5	10.11±0.15	25.54±0.3.17	14.52±3.24	30.11±0.16	80.28±0.07
RP6	8.77±1.24	18.18±2.81	13.29±0.11	20.2±4.24	60.44±1.00
RP7	ND	2.51±0.28	ND	2.63±0.05	5.14±0.33
RP8	1.42±0.05	47.02±4.70	9.22±0.77	158.83±10.66	216.48±16.16
RP9	2.31±0.01	28.13±2.83	2.66±0.31	16.12±1.93	49.2±1.21
RP10	0.71±0.00	1.15±0.50	ND	ND	1.86±0.50

ND=Not Detected,or below detection limit (0.5µg/kg)

From 10 collected samples of roasted peanut, all showed aflatoxin contamination ranging from 1.86 µg/kg to 216.48 µg/kg having mean value 58.78µg/kg ± 75.56 *SD* (Median 17.07µg/kg)

which showed a great variability of aflatoxin contamination among samples as the samples have larger standard deviation than mean value. The concentration range and mean value of aflatoxin in this study showed greater difference from survey conducted in Korea (Mehrdad *et al* ,2011) having range of 2.00–28.24 μg/kg and mean of 12.30 μg/kg ± 67*SD*. There is also a significant difference in mean value of AFB1 (the most potent type of aflatoxin) between samples of this study (43.86 μg/kg ±61.07 *SD*) and finding of similar author Mehrdad *et al* (2011) (7.97 μg/kg ± 7.75 *SD*). Chilaka (2012) from Guinea also reported mean value of AFB1 upto 6.1μg/kg with median 1.9μg/kg and all samples were below 20μg/kg interms of total aflatoxin, which is very smaller contamination compared to the present study.

Similarily, Yousif *et al* (2010) reported less contamination of roasted peanut in Sudan with percentage of 11% than present study. But, he confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2 which agrees with the present study. It is clearly observed that result of this study ,in both mean value and contamination ranges, showes higher contamination than specified surveyes; the reason may be due to less knowledge among processors and absence of regulatory limit regarding aflatoxin in our country.

In other study by Freitas *et al* (1998) of Brasil, upto 1789μg/kg of AFB1 was detected in peanut and peanut products including roasted peanut which showes higher contamination than this study. In the same country, the incidence was reported upto 52% by carlos *et al* (2009) . However, after implementation of tolerance limit for aflatoxin in Brazilian food stuffs including peanut and peanut product, the incidence had significantly minimized to a 39.6% ; with all samples of roasted peanut below detection limit. From both authors, it could be understood that the higher contamination of aflatoxin in roasted peanut could arise from absence of standard or regulatory limit by government and Good Manufacuring practice among processors of peanut which could be applied to our case, Ethiopia.

However, the result of this investigation is inline with result of Zhang *et al* (1996) , from Asia who reported AFB1 from 1 to 244μg/kg from less than half percent of total samples.

Investigation out put of this study revealed that all of the samples collected for analyses were contaminated with AFB1, AFG1, AFB2 and AFG2 to the levels of 90%, 90%, 80% and 60% respectively; found in the order of the relative potency of the four aflatoxin types as assessed in

ducklings, AFB1 > AFG1 > AFB2> AFG2 (Leo, 2013 ; Omer, 2001). These contamination levels were found to be more greater than similar studies as discussed above. This evidence leads us to say peanut from Ethiopian farmers was highly contaminated and can be confirmed by study of Eshetu (2010) from Ethiopia, who studied contamination level of aflatoxin in raw peanut from Ethiopian farmers and reported upto 73% of samples were contaminated between 0.57μg/kg to 447.02μg/kg (AFB1 up to 324.34μg/kg).

Apart from contamination of raw peanut, however, the high cost of testing discourages the majority of the farmers, processors and traders from testing their products. This setback, in the absence of adequate knowledge, is further compounded by the reluctance of consumers to pay the extra cost of a tested product, when there is a readily available alternative (Mutegi *et al*, 2013).

4.3. Evaluation of aflatoxin results against different international standards

The samples included in this investigation have been analyzed under reliable method (as verified in the methodology), HPLC, and the results were evaluated under different internation standards for the rejection percentage as detailed in the table below. The evaluatin of results against different international standards included only local samples so as to ease evaluation and generalize quality of peanut butter from Addis Ababa markets.

Table 11: Percentage of rejected local samples due to aflatoxin (μg/kg) under different internation standards.

Standard	EU		Codex		WHO/FAO	EAS	
product	Total AF >4	AFB1 >2	Total AF >10	AFB1 >5	Total AF >30	Total AF >15	AFB1 >5
Peanut butter	91%	93.33	84.44%	88.89	60%	77%	88.89
Roasted Peanut	90%	90%	70%	90%	40%	50%	90%
Weghted mean	90.82%	92.73 %	81.82	89.09 %	56.36%	72.09%	89.09 %

All values within the same row are cumulative, thus do not addup to 100%.

The categories of investigated samples were peanut butter and roasted peanut. The reason to compare results of these samples against EU, Codex and WHO/FAO is to see the percentage of rejected samples or to summarize the percentage of samples that fits for human consumption from Addis Ababa market in terms of aflatoxins contamination. Due to the absence of local standard on aflatoxin, the study results were evaluated against the standards of aforementioned internationally recognized bodies.

More than 91% of Peanut butter samples sold in Addis Ababa supermarkets and retailers could be rejected under European Union (EU) standard, if these products were exported to European countries. Moreover, upto 60% of peanut butter samples could be rejected under WHO/FAO standard.

When both categorized samples, peanut butter and roasted peanut, were evaulated by a single mean (Weghted mean), more than 90% samples could be rejected under EU. Relatively, minimum rejection would be recorded under WHO/FAO, if both samples were marketed in countries using WHO/FAO standard. Maximum rejection of samples under EU is due to stringent standard of EU (≤4μg/kg).

When local peanut butters were compared against East African Standard (EAS), 77% of products were rejected for total aflatoxin content and 89% of products did not conform to the specification of AFB1. In addition, 90% of samples of roasted peanut collected under this investigation did not comply with the EAS (≤5μg/kg) for its AFB1. As a cumulative report, 89% of peanut products (peanut butter and roasted peanut) traded in Addis Ababa market were highly contamicated and could not fit for human consumption under East African Standard (EAS).

Moreover, this study has found that 84.44% (Table 11) of peanut butter samples have aflatoxin value exceeding 10μg/kg of codex limit where as Mutegi *et al* (2013) from Kenya reported upto 69% which is less than finding of this study. The same author reported percentage of aflatoxin contamination from roasted peanut collected from Nairobi exceeding 10μg/kg was upto 56.2% which is still far less than finding of this study (70%). The higher percentage in contamination may arise from absence of regulatory limit of aflatoxin in Ethiopia, but kenya has the regulatory limit on aflatoxin.

Samples of Peanut butter and roasted peanut from Guinea have fulfilled ≤20μg/kg criterion of FDA by 100% and 85 % respectively (chilaka,2012) which is below results of this study. The

practice of standard implementation and food safety training on peanut butter for cottage industries may be the reason for existed difference between two countries.

According to Eshetu's (2010) finding from Ethiopian farmers, newly harvested peanut has lower aflatoxin contamination; upto 83.33 % and 91.67 % of his samples collected from Ethiopian farmers were below 4 ppb and 20 ppb respectively. Contrarily from one year stored samples only 4 % and 52 % were below the aforesaid international standards. According to his investigation, raw peanuts were less contaminated at the farm point, but very contaminated after longer handling. The present study concluding most of these infested samples where used by processors and this in turn led to contamination of more than 90% of peanut butter and ready to eat roasted peanuts by aflatoxin (considering that peanut butter and ready to eat roasted peanut are sourced from this farmers). This variation in aflatoxin might happen due to poor storage and transportation system which support the growth of aflatoxin producing fungi. Mehrdad *et al.* (2011) also justified such contamination of aflatoxin that occurred after harvest could be due to lack of regulations or poor enforcement, which make the use of such contaminated commodities inevitable.

During this investigation, all brands of peanut butter obtained during sampling were included in the study. Evethough samples were randomly picked from supermarkets and shops, brand inclusion was the major concern throughout the research timeframe. However, there might be brands not included in the study which existed during study. In addition, only Supermarkets and shops found in Addis Ababa were included in sampling plan. Other brands of peanut butter may exist in Surrounding towns of Addis Ababa city. Thus, this study hopes next coming works will complement the this job through inclusion of all towns and cities of Ethiopia to generally speak about quality of Ethiopian peanut butter and other peanut products.No imported roasted peanut has been obtained in any market point.

4.4. Investigation results on *Salmonella*

4.4.1. Local peanut butter

This survey was conducted to obtain information on the presence of pathogen in peanut butter currently available in the market of Addis Ababa. In this regard, the study aimed at investigating the presence of *Salmonella* in different brands of peanut butter traded in Addis Ababa markets.

Accordingly, 48 samples of peanut butter were randomly collected from supermakets and shops and analyzed at Bless Agri Foods Laboratory PLC. Out of 48 samples, 45 samples were from local producers and the remaining 3 samples were imported product. From total of 45 local samples, *Salmonella* was detected in 3 samples of peanut butter (Table 12) and *Salmonella* was absent in the remaining 45 samples.

The reason to investigate pathogenes specifically *Salmonella* was that there have been different reportes from different countries that indicated presence of *Salmonella* in low moisture foods commonly in peanut butter. The tolerance limit of *Salmonella* in ready to use therapeutic foods (RUTF) should be zero per 125g. Its contamination could be happen, as general, due to absence of good manufacturing practices and good hygienic practices among food processors

Table 12: Analyses results of *Salmonella* in 45sampled peanut butter from local producers

No.	Sample code	Result
1	PB1- PB3	Not detected
2	PB4	Detected
3	PB5- BP19	Not detected
4	PB20	Detected
5	PB21- PB30	Not detected
6	PB31	Detected
7	PB32 – PB45	Not detected

Low-moisture products such as peanut butter have been implicated in outbreaks of salmonellosis as highlighted in different studies. According to invetigation result of this study (Table 12), it is imperative to know how such products in our market are contaminated with pathogenes (*Salmonella*). As different standards limit *Salmonella* to zero level for human consumption, 3 detections out of 45 samples may indicate the presence of poor manufacturing practices among processors. In addition, based on the specific strain of *Salmonella*, it might have affected the consumers of the products as small amount can leads to human illness. These three detection may be meaningful when refered to the report of Yuhuan (2009). The report said, in the 2006-2007 outbreak associated with peanut butter, *Salmonella* was found at 1.5 MPN/g (Most Probable Number per gram) in an unopened jar and a lower level was found in another product sample. Infection has occurred from consuming low-moisture products contaminated with less than 1 cfu/g depending on the host, the product, and the *Salmonella* strain (Yuhuan, 2009).

The samples, in which those detection of salmonella were recorded, have higher aflatoxin concentration (93.16μg/kg, 242.71μg/kg and 265.34μg/kg). Where there was higher aflatoxin contamination, there was also contamination of *Salmonella*. This indicates lack of understanding of any quality criterion among processors and confirming that under what circumstances these producers were processing their products.

Hence, these three samples can talk more about severity of problem and indicating: poor sanitation practices, poor equipment design, improper maintenance or poor ingredient control etc in those facilities of small scale processors found in Addis Ababa. According to Neilh (2009) , PEM (2009) and Lindhardt (2009), the concequence of such probrem ended up by various food borne illness outbreaks and food recalls because of actual or potential contamination cuased by *Salmonella sp* in peanut butter product occurred during the year 2008 in USA , and resulted in more than3, 900 food products being recalled. When come to Ethiopia, how many outbreaks have been appeared and recorded in this regard(as peanut butter processors are appearing at alarming rate)? Unkwown! Might any salmonella related diseases come due to consumption of peanut butter yet? No research based data is available so far! Tracing such cases to the main causes is very difficult or controversial. But the present study would be saying there might be people whose health were affected through consumption of contaminated peanut butter, and necessitate the implementation of Good Hygiene practice and HACCP principle at processors premises, strong regulation by government. If possible processors should: analyze *Salmonella* in their products as part of quality assurance; establish "high hygiene" zones with more stringent hygiene requirements and procedures, and analyze the air systems as dust is one factor for contamination of salmonella. According to Powell (1998), cros-contamination between raw goods and finished goods should be controlled because it is a significant contributing factor in 32.1% of the cases.

Fig 5: Typical colony characteristics observed in Simmon's citrate agar (deep blue color) and MR-VP agar (pink to red color in addition of methyl red).

There are considerable data on outbreaks of salmonellosis in different countries. But, there are a very limited number of researches found to see the incidence of salmonella contamination in peanut butter against this study.

Upcoming works may fill the gap of this study through analyzing most pathogenic microbial parameters and covering all products of peanut butter produced throughout the country so as to see the microbiological quality of peanut butter produced in Ethiopia.

4.4.2. Imported peanut butter

There were limited numbers of peanut butter brands in Addis Ababa markets. From total of 48 collected samples from the market, only 3 imported brands were found during sampling period.

Table 13: Analyses result of *Salmonella* in imported peanut butter samples

Code of samples	Result
PB46	Not detected
PB47	Not detected
PB48	Not detected

Analyses of *Salmonella* was conducted for the 3 samples and *Salmonella* was not detected (Table 13). Due to very limited number of imported peanut samples, comparison with local products interms of salmonella percentage contamination was found to be not feasible. However, *Salmonella* was absent in the three samples because this products might come from countries where stringent follow up of pathogens is implemented, unlike local production system.

4.5. Description of quality of peanut butter in terms of oil separation

4.5.1. Local peanut butter

The extent of surface oil is explained by the word "very noticeable" (borrowed from EAS document) if the surface oil is very visible and could be flow or poured. Accordingly, out of 45 local samples collected from markets of Addis Ababa , 11 samples showed oil separation. According to Ethiopian Food Corporation (1998), the use of processed foods such as peanut butter in Ethiopia is increasing. The most serious problem of natural peanut butter is the tendency of the oil to separate during storage as it has negative impact on purchase and general quality of peanut butter.

Table 14: Local samples of peant butter that showed oil separation

Sample code	Oil separation
PB2	Very noticeable
PB4	Very noticeable
PB5	Very noticeable
PB10	Very noticeable
PB20	Very noticeable
PB23	Slightly noticeable
PB25	Very noticeable
PB27	Very noticeable
PB31	Very noticeable
PB38	Slightly noticeable
PB43	Very noticeable

The reason to oil separation may show inapropriate preparation or due to extra added oil. Flor *et al.* (2006) explains output of such problem as; oil separation in peanut butter may develop rancidity during storage and gives off-flavor at the point of tasting. According to different literatures, deterioration of peanut butter can arise from oxidative rancidity that develops in the unsaturated portion of oil when it is exposed to air as it was observed in above samples of this study. Thus, oil separation can potentially be converted to rancidity. However, due to scope limitation and budget constraints, this study didnot encompase the analyses of free fatty acids and peroxide value to see the degree of rancidity.

Similar studies conducted in Argentina and China indicated that peanut butter of both countries showed higher free fatty acid percentages and peroxide values compared to U.S. peanut butter (SPG, 2009)

When looking further into the previous aflatoxin results and oil separation, both have a common point of interception. Meaning, there were 7 top samples of peanut butter showing maximum aflatoxin contamination (>200μg/kg) and 5 of them had showed oil separation. This simply implies to the logic that manufacturer of those samples did not know how to select good quality peanut and how to prepare the product. High aflatoxin contamination along with oil separation makes the product risky for consumption.

Quality criteria and controlling mechanism on those product should be implemented by government to protect health of consumers. From manufacturer's side, they should first understand how to deliver good products to the market for the sake of healthy community. They have to adopt HACCP principles to their premises so as to minimize risks related to product they produce. In the absence of quality criteria, any food product may not be safe for consumption. In actual sense, intervation is necessary in this regard.

4.5.2. Imported Peanut butter

In three samples of imported peanut butters, there was no observed oil separation. Samples of imported peanut butter have a very good spread as its oil was not separated from the remaining paste. The absence of oil on the surface of the paste gives the guarantee that the product can be stored relatively longer duration without rancidity when compared to local products.

4.6. Standards on Aflatoxin and *Salmonella*

Different countries have their own standards regarding any thing. Food standard is one of the most important standard category for many countries. There are internationally used or accepted food standards prepared by UNICEF, WHO, FAO, WFP, codex etc. Different countries may adopt standards from those international organizations or prepare their own ones. Table 15 indicates different standards on aflatoxin. Regarding *Salmonella*, most countries of the world use standards of Codex which indicates absent/125g.

Table 15: Some Internation and national Pemissible limits for aflatoxin (μg/kg) (as an example)

Country	Total Aflatoxin (Max)	AFB1 (Max)	Reference	Remark
EU	4μg/kg	2	PACA, 2012; Judy, 1998	Sorghum and others
WHO/FAO	15		Bhat *et al.*, 1996	Groundnut ready to eat
	30		Moss, 1996	Any food for human consumption
COdex	10	5	Codex 2001	In processed peanut
FDA	20		Chilaka (2012)	All kind of foods
USA	20		(PACA, 2012)	Sorghum and other foods
India		30	Bhat *et al.*, 1996	Groundnut ready to eat
Kenya	10	5	Ndung'u *et al*, (2013), (Mutegi *et al*, 2013,	Peanut butter and others
Zimbabwe	20	5	Siwela, 1999	Any food for human consumption
South Africa	10	5	MRC policy brief	In all food
Papua New		10	Chilaka (2012)	
East Africa Standard	15	5	EAC,2013	Peanut butter
Ethiopia	?	?		?

As a precautionary measures, countries should have standards to control the quality and safety of food items. The rewards of this act will protect the health of consumers. Citizens could potentially be utilized when their health is protected against any pathogenical and mycotoxical risks. Hence, health should be a prime issue! In this regard, food produced locally and/or imported from abroad must be free of any hazards. Because, chemical, physical and microbiological hazards are frequently affecting different segment of socities. Specially *Salmonella* from microbes and aflatoxin from chemical toxins are being the causes of human illness as highlighted in different literatures. According to specification of UNICEF and MSF (Medecins Sans Frontieres) on RUTF , for example, *salmonella* is the most serious or highest priority microbes recieving a great attention in ready-to-use lipid based therapeutic and supplementary foods. From chemical toxin, aflatoxin is a primary quality requirement in RUTFs.

Recalling Hilina's case (Ethiopia), both aflatoxin and *salmonella* in RUTF were claims of contamination raised from its customers: international NGO's like UNICEF and MSF. The first claim was product (one batch of Plumpy'nut) contamination by aflatoxin above permissible limit of the customer's specification(<5μg/kg). To resolve the problem, detailed investigation was performed by company and the cause for contamination was identified. Different sorting machines were installed in peanut process line. To minimize the potencial cause of contamination, farmer level training was held for peanut producers. The training by Hilina had also addressed those stakholders playing a role on value chain of peanut butter. The second claim by customers was regarding *salmonella* issue. *Salmonella* was detected in one batch of plumpy'nut. To investigate the problem, the factory ceased production for minimum of 4 months. The investigation spectra were wide due to nature of *Salmonella*. Detailed corrective actions including hygiene sufficiencycy, cross contamination , processing points, storage adequacy, equipment cleanliness, and employees training were taken to solve the problem.

In general, the problems highlighted here are showing that contamination of pathogens and mycotoxins may escape and exist even in sophesticated and system certified food manufacturing plants. In this regard, what about quality of food products processed under small scale processors? Problems appeared in Hilina might similarily be happened in those small scale peanut butter processors. The only means to know is through regulations by government. All the

issues discussed here is to emphasis that there are an alarming problems in food items specially manufactured under small scale processors. Studies revealing that uncontrolled exposure of consumers by pathogens like *salmonella* and mycotoxins like aflatoxin are common in Ethiopia. But, no standard is available in Ethiopian Standard Agency regarding Aflatoxin and *Salmonella* in peanut butter. According to information from Ethiopian Food, Medicine and Health Care Administration and Control Authority, no standard is available regarding *salmonella* and Aflatoxin. The authority has informally using codex standards for microbiological parameters and aflatoxin. No specific limit for aflatoxin. Any absence of experience on aflatoxin by the authority meaning no inspecton has been conducted regarding aflatoxin in peanut and peanut products.

However, even if there is no standard of peanut butter on aflatoxin and *salmonella* in Ethiopia, East African community (EAC) has jointly established standard on aflatoxin in peanut butter. This established standard or specification of peanut butter showed that maximum acceptable total aflatoxin in peanut butter shall be $\leq$ 15μg/kg and for AFB1 shall be 5μg/kg (Table 15). In addition, the standard stated that peanut butter shall be free of pathogenic organisms and shall conform to the microbiological requirements (*moulds* max 10^3 /g and *E.coli* zero per gram of sample). The standard did not specify *Salmonella*, but stated the absence of pathogens as a general. In this case, Ethiopia had participated in establishing the standard. The tricky issue here is Ethiopia did not adopt the standard to the existing local situation. Thus, no standard or regulatory limit on peanut and peanut products has been put for both Aflatoxin and *Salmonella* in Ethiopia.

According to information obtained from Ethiopian Standard Agency, however, African Regional Standards Organization (ARSO) adopted different standards related to aflatoxins in different food products which will be part of Ethiopian standards. Meaning, about 13 types of standards are in progress to be adopted by Ethiopian Standards Agency.

5. Conclusion and Recommendation

5.1. Conclusion

According to investigation conducted, 93% of samples of peanut butter products traded in Addis Ababa markets had total aflatoxins ranged from 3.92μg/kg to 547.85μg/kg, with mean total aflatoxin of 98.24μg/kg ± 111.18 *SD*. From this, 91%, 84% and 77% of samples showed concentrations over the tolerance limit established by European union, codex and East African Community, respectively. In addition, Peanut butter samples showed the highest AFB1 concentration ranged from 2.65μg/kg to 270μg/kg with mean value of 51.94 μg/kg ± 65.33 *SD*. Maximum rejection of samples in AFB1 was recorded under EU regulatory limit up to 91% followed by Codex and EAS (89%). The analysis was conducted using HPLC technique.

All samples of Ready to eat roasted peanut collected from street vendors of Addis Ababa city were contaminated by total aflatoxin ranged from 1.86 μg/kg to 216.48 μg/kg having mean value 58.78 μg/kg ± 75.56 *SD*. The potent AFB1 was detected in samples upto maximum value of 158.83 μg/kg with mean value 43.86 μg/kg ± 61.07 *SD*. Roasted peanut sold by street vendors have relatively smaller aflatoxin contamination than peanut butter, because sellers of the roasted peanuts use comparatively good peanut than processors of peanut butter as it could visually be checked during consumption of roasted peanut.

Most peanut butter brands and roasted peanut contain aflatoxin well in excess of the maximum levels laid down by different internationally recognized bodies. So, the level of aflatoxin contamination obtained in this study is very serious as far as human safety is concerned. Aflatoxin B1 (AFB1), which is a highly toxic, mutagenic, carcinogenic and immunosuppressive compound, has occured in higher contamination range in 91 % of the samples. It implies that people who consume these samples could develop health complication and hence this intoxication may impose primarily liver cancer to them or synergistically act up on liver with Hepathesis B virus. Thus, contamination of peanut butter by AFB1 would be a major food safety concern in those products produced in cottage industries of Addis Ababa, for the reason that of its adverse effect on human and animal health. The results indicated that aflatoxin

contamination of peanut products could be a public health concern in Ethiopia, since it contributes to the general human exposure to these toxins, especially among children.

Analyses of *Salmonella* have revealed that 3 samples of peanut butter showed detection. As *Salmonella* can cause illness, three samples of detection is significant as far as food safety is considered. The result of the study has suggested that cross contamination plays a major role for presence of *Salmonella* in the products. Cross contamination is the transfer of bacteria from one surface, object or place to another. Limited exposure of processors to the technical knowledge and general awareness of food safety could also be one factor for production of unsafe products.

In relation to general quality of peanut butter, specifically oil separation, 24% of samples under study have showed oil separation to an extent can potentially cause rancidity. Oil separation in peanut butter may develop rancidity during storage and gives off-flavor at the point of tasting. Consequently, deterioration of peanut butter can arise from oxidative rancidity that develops in the unsaturated portion of oil when it is exposed to air. This problem substantiated the use of improper processing method, absence of knowledge, or wrong formulation.

In due course of research, it was observed that where there was high oil separation, there was also higher aflatoxin contamination noted in the same samples; it may be deduced that there could be peanut butter processors who engaged in the process with no understanding of quality criteria.

Ethiopia has no standard on Aflatoxin and *Salmonella* so far. Those bodies that are in charge of regulating and controlling food processors have no means to blame the processors of peanut butter for low quality product. Standard is very important for peanuts and peanut butter as they are inexpensive sources of protein. The existence of regulatory and controlling acts is very important in minimizing the incidence of food contaminations. For instance, due to absence of regulatory systems among cottage industries, higher aflatoxin contamination was recorded than most results indicated in various survey reports.

5.2. Recommendation

High aflatoxin contamination at market level especially in peanut and peanut products implies that the prevailing post-harvest handling practices are insufficient in controlling contamination and in some cases, have worsened contamination levels. On top of this, the absence of awareness about aflatoxin and inefficient technical knowledge on how to produce among most cottage processors worsen the problem. Hence, several cultural practices such as sorting, sieving, winnowing and drying should be practiced by the traders and processors as ways of reducing aflatoxin contamination from peanuts to be sure only good, wholesome kernels are used in peanut butter.

The bacteriological quality of traditionally processed peanut butter creates a potential danger with regard to public health. As such, the isolation of organisms like *Salmonella* sp, which are of public health significance in traditionally processed peanut butter, could pose health hazards to consumers. Therefore, processors of the product should take care of cross contamination during production and should implement HACCP principles to their process line.

There is a need for awareness creation at all levels of the peanut value chain, especially for end consumers, campaigns to raise consumer demand for safe, high-quality food.

Comprehensive, multi-sectoral approaches are required to reduce and control aflatoxin prevalence and exposure in Ethiopia.

Unless strict monitoring measures for aflatoxin and pathogenic microbes are put in place, condemned nuts will continue to be available in the markets for human consumption. Hence, Quality criteria and controlling mechanism on those products should be implemented by regulatory bodies to protect health of consumers. Education of the local producers by regulatory bodies on the use of the hazard analysis critical point (HACCP) concept from the raw material through processing stages to storage and /or retailing is advocated in view of possible microbial and mycological hazards in traditionally processed peanut butter. There is a need for systematic and universally applicable approach to food safety control; enforcing proper sanitation and monitoring of products by relevant regulatory bodies. Intervation is necessary in this regard!

Traders and manufacturers are obligated by law to countercheck the safety and quality of food products they manufacture, sell, distribute, market or import into the country. Meaning, they should practice to test their products by themselves or via sending their samples to available analytical laboratories for verifying the compliance of safety and quality of their products. In this regard, affordable costs for raw materials and product analysis should be given an attention!

Wider investigations regarding peanut butter produced all the way through Ethiopia are required to precisely generalize the prevalence of the aflatoxin and salmonella in the country. Furthermore, study of other serious pathogens in food items, degree of aflatoxin intoxication among consumers (risk assessment studies); unlike HPLC, low cost, rapid and easy methods for aflatoxin determination could be the next works to be handled by upcoming researchers which this study did not address.

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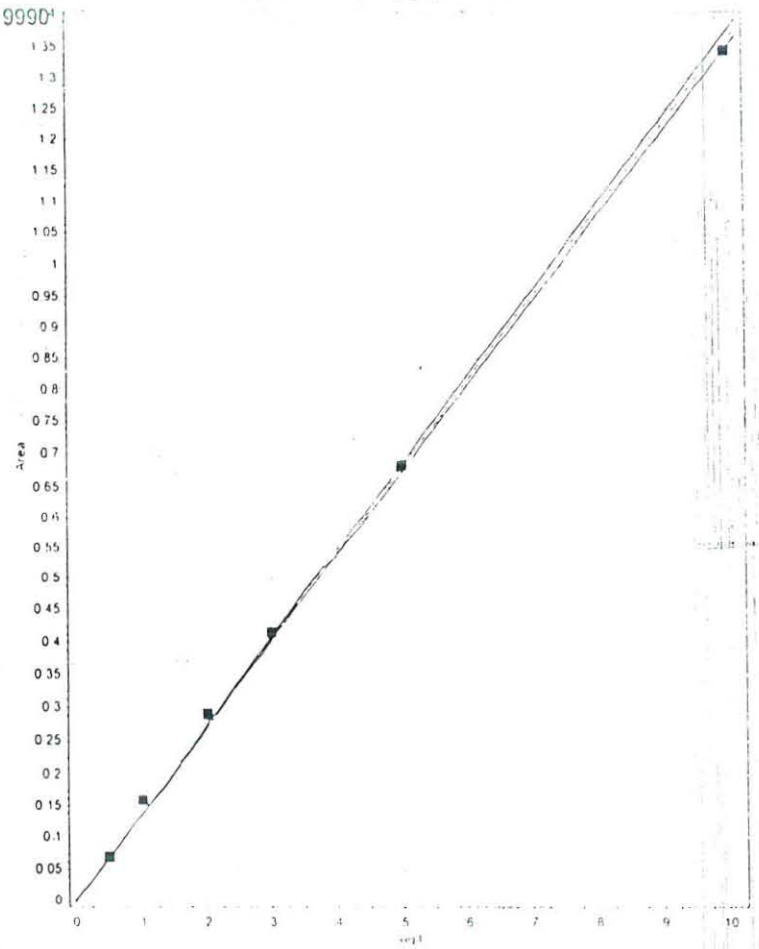
Appendix A

Average results of aflatoxin from local peanut butter samples

No.	Code	Result (µg/kg)				
		G2	G1	B2	B1	Total
1	PB1	ND	ND	ND	ND	ND
2	PB2	5.34	135.18	10.34	135.13	285.99
3	PB3	3.02	22.7	1.91	18.79	46.42
4	PB4	4.29	123.52	4.88	110.02	242.71
5	PB5	1.01	8.25	4.25	26.6	40.1
6	PB6	2.78	30.96	2.07	18.75	54.57
7	PB7	3.86	39.61	3.56	26.53	73.56
8	PB8	0.59	7.22	ND	4.29	12.1
9	PB9	7.9	247.1	21.81	270.04	547.85
10	PB10	1.06	22.96	2.06	17.71	43.79
11	PB11	7.22	87.05	10.64	72.3	177.2
12	PB12	4.9	41.33	5.77	27.68	79.68
13	PB13	6.67	122.78	15.8	137.22	282.47
14	PB14	6.87	64.14	7.98	43.35	122.34
15	PB15	1.29	9.65	0.95	5.55	17.44
16	PB16	0.79	5.51	0.76	3.4	10.46
17	PB17	4.56	43.55	6.14	39.64	93.9
18	PB18	1.14	6.37	10.06	8.04	25.61
19	PB19	2.36	24.19	3.95	24.05	54.55
20	PB20	3.36	41.11	6.8	41.89	93.16
21	PB21	4.83	80.26	5.03	43.36	133.48
22	PB22	5.03	44.57	7.09	58.69	115.38
23	PB23	2.71	7.15	4.48	9.22	23.56
24	PB24	3.11	35.76	3.89	23.16	65.92
25	PB25	3.14	94.19	10.34	172.07	279.74
26	PB26	ND	2.8	1.93	12.8	17.53
27	PB27	0.5	4.33	2.81	18.57	26.21
28	PB28	1.36	1.64	10.55	110.63	124.18
29	PB29	ND	1.27	ND	2.65	3.92
30	PB30	ND	1.33	12.93	37.61	51.87
31	PB31	0.74	17.21	8.65	238.74	265.34
32	PB32	0.75	4.77	2.48	10.79	18.79
33	PB33	ND	4.08	2.43	5.98	12.53
34	PB34	ND	0.87	1.03	7.71	9.7
35	PB35	ND	ND	ND	ND	ND
36	PB36	ND	ND	0.67	3.04	4.2
37	PB37	ND	ND	ND	ND	ND
38	PB38	ND	15.81	8.05	191.59	215.45
39	PB39	2.85	27.95	6.35	50.57	87.72
40	PB40	0.95	7.01	3.96	25.61	37.53
41	PB41	ND	1.28	2.06	18.04	21.38
42	PB42	ND	2.53	1.19	7.03	6.07
43	PB43	3.76	7.56	2.08	13.36	26.76
44	PB44	9.13	46.59	7.28	29.12	92.12
45	PB45	13.04	100.87	8.89	60.06	182.86

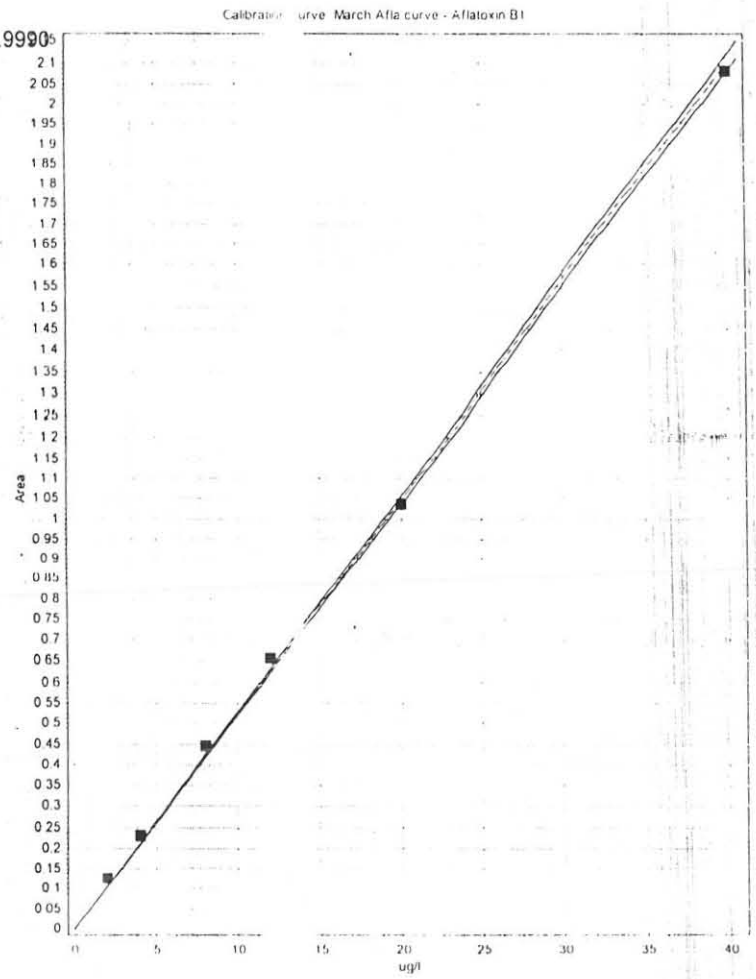
= 0 13560
Correlation Coef 0 9990
Weighting None
Force zero Yes



Calibration table Aflatoxin B2

#	Used	Name	ug/l	Area	RF	Chromatogram	Date	Area (recalc)	Res %	time [Min]	Le
1	1	Point 1	0 50	0 07	7 19	Aflatoxin Standard 5ppb1 (1)	3/12/2014 3 08 44 PM	0 07	2 45	7 84	
2	1	Point 2	1 00	0 16	6 29	Aflatoxin Standard 1oppb1 (1)	3/12/2014 3 17 13 PM	0 14	14 75	7 84	
3	1	Point 3	2 00	0 29	6 88	Aflatoxin Standard 20ppb1 (1)	3/12/2014 3 48 02 PM	0 27	6 73	7 83	
4	1	Point 4	3 00	0 42	7 22	Aflatoxin Standard 30ppb1 (1)	3/12/2014 3 49 05 PM	0 41	2 13	7 84	
5	1	Point 5	10 00	1 34	7 44	Aflatoxin Standard 100ppb2 (1)	3/12/2014 3 [illegible] 43 PM	1 36	0 86	7 81	
6	1	Point 6	5 00	0 68	7 32	Aflatoxin Standard 50ppb2 (1)	3/12/2014 4 [illegible] 35 PM	0 68	0 74	7 85	

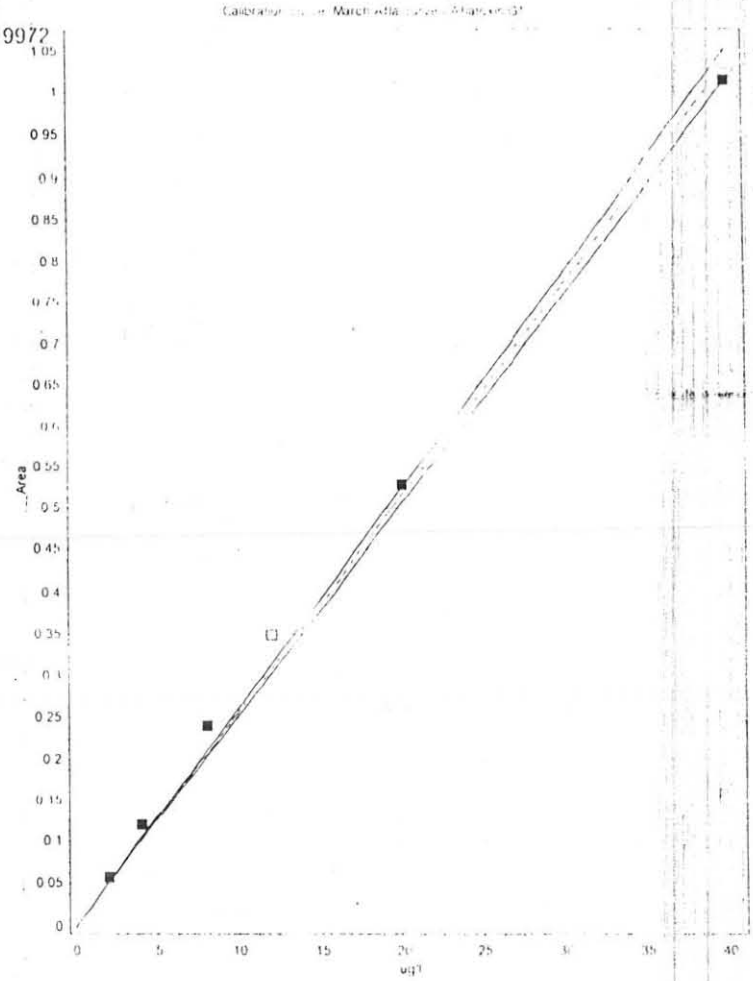
b = 0.05231
Correlation Coef. : 0.9990
Weighting : None
Force zero : Yes



Calibration table : Aflatoxin B1

#	Used	Name	ug/l	Area	RF	Chromatogram	Date	Area (recalc)	Res %	time [Min]	Level
1	1	Point 1	2.00	0.13	15.75	Aflatoxin Standard 5ppb1 (1)	3/12/2014 3:08:44 PM	0.10	17.59	9.17	1
2	1	Point 2	4.00	0.23	17.45	Aflatoxin Standard 10ppb1 (1)	3/12/2014 3:17:13 PM	0.21	8.71	9.18	2
3	1	Point 3	8.00	0.45	17.97	Aflatoxin Standard 20ppb1 (1)	3/12/2014 3:48:02 PM	0.42	6.00	9.16	3
4	1	Point 4	12.00	0.66	18.24	Aflatoxin Standard 30ppb1 (1)	3/12/2014 3:49:05 PM	0.63	4.59	9.20	4
5	1	Point 5	40.00	2.08	19.23	Aflatoxin Standard 100ppb2 (1)	3/12/2014 3:50:43 PM	2.09	0.60	9.15	6
6	1	Point 6	20.00	1.04	19.30	Aflatoxin Standard 50ppb2 (1)	3/12/2014 4:55:35 PM	1.05	0.96	9.20	5

b = 0.02578
Correlation Coef 0.9972
Weighting : None
Force zero : Yes



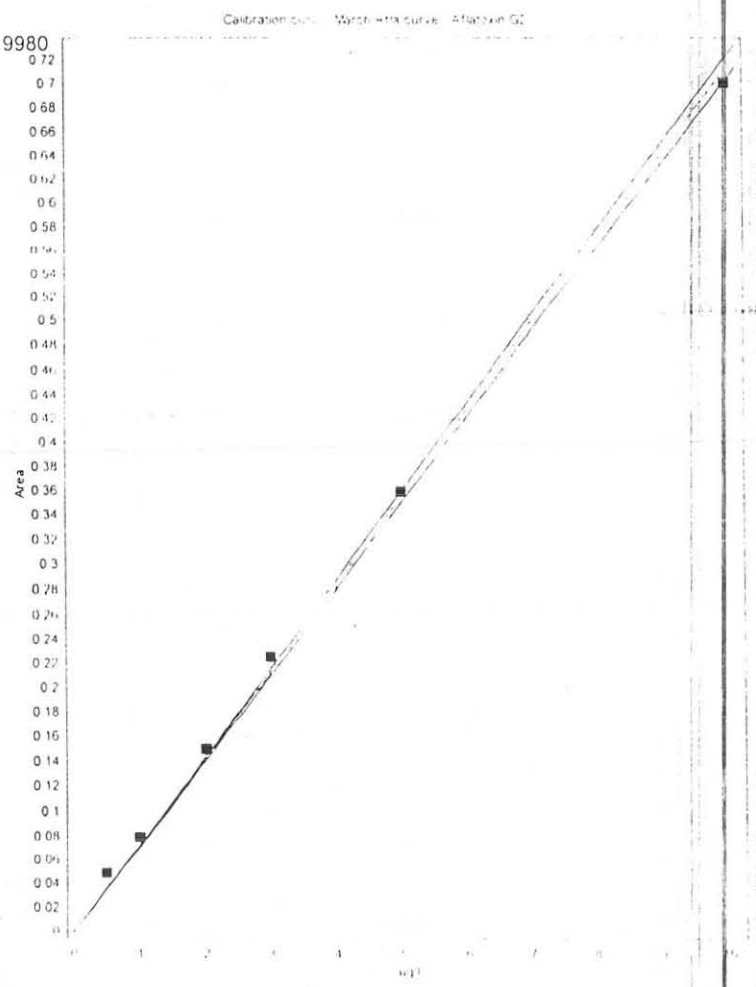
Calibration table : Aflatoxin G1

#	Used	Name	ug/l	Area	RF	Chromatogram	Date	Area (recalc)	Res %	time [Min]	eve
1	1	Point 1	2.00	0.06	34.42	Aflatoxin Standard 5ppb1 (1)	3/12/2014 3:08:44 PM	0.05	11.28	7.00	
2	1	Point 2	4.00	0.12	33.79	Aflatoxin Standard 1oppb1 (1)	3/12/2014 3:17:13 PM	0.10	12.90	7.01	
3	1	Point 3	8.00	0.24	33.23	Aflatoxin Standard 20ppb1 (1)	3/12/2014 3:48:02 PM	0.21	14.34	6.99	
4	0	Point 4	12.00	0.35	34.24	Aflatoxin Standard 30ppb1 (1)	3/12/2014 3:49:05 PM	0.31	11.74	7.01	
5	1	Point 5	40.00	1.02	39.38	Aflatoxin Standard 100ppb2 (1)	3/12/2014 3:50:43 PM	1.03	1.50	6.98	
6	1	Point 6	20.00	0.53	37.85	Aflatoxin Standard 50ppb2 (1)	3/12/2014 4:55:35 PM	0.52	2.45	7.02	

Calibration Report :
File : March Afla curve

Component Aflatoxin G2

Polynom y = b x +a
a = 0
b = 0.07108
Correlation Coef 0.9980
Weighting None
Force zero : Yes



Calibration table . Aflatoxin G2

#	Used	Name	ug/l	Area	RF	Chromatogram	Date	Area (recalc)	Res %	time [Min]	Lev
1	1	Point 1	0.50	0.05	10.06	Aflatoxin Standard 5ppb1 (1)	3/12/2014 3:08:44 PM	0.04	28.51	6.09	
2	1	Point 2	1.00	0.08	12.52	Aflatoxin Standard 1oppb1 (1)	3/12/2014 3:17:13 PM	0.07	10.97	6.09	
3	1	Point 3	2.00	0.15	13.26	Aflatoxin Standard 20ppb1 (1)	3/12/2014 3:48:02 PM	0.14	5.71	6.08	
4	1	Point 4	3.00	0.23	13.28	Aflatoxin Standard 30ppb1 (1)	3/12/2014 3:49:05 PM	0.21	5.61	6.09	
5	1	Point 5	10.00	0.70	14.26	Aflatoxin Standard 100ppb2 (1)	3/12/2014 3:50:43 PM	0.71	1.35	6.08	
6	1	Point 6	5.00	0.36	13.89	Aflatoxin Standard 50ppb2 (1)	3/12/2014 4:55:36 PM	0.36	1.30	6.09	