ST. MARY'S UNIVERSITY

SCHOOL OF GRADUATE STUDIES

INSTITUTE OF QUALITY AND PRODUCTIVITY MANAGEMENT

DETERMINATION OF MYCOTOXIN LEVELS IN PAPRIKA POWDER/RED PEPPER FOR CONSUMPTION AND EXPORT PURPOSES: IN ADDIS ABABA CITY ADMINISTRATION.

BY: KALEELIAS AGMUAS

ADVISOR: ABDU ABAGIBE (PhD)

JUNE 2022

ADDIS ABABA, ETHIOPIA

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A THESIS SUBMITTED TO ST. MARY'S UNIVERSITY, SCHOOL OF GRADUATE STUDIES, INSTITUTE OF QUALITY AND PRODUCTIVITY MANAGEMENT IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN QUALITY AND PRODUCTIVITY MANAGEMENT

JUNE 2022
ADDIS ABABA, ETHIOPIA

DECLARATION

I hereby declare that this thesis is my own work towards the award of MSc degree and that to the best of my knowledge and wherever others‟ ideas or words have been included, I have adequately cited and referenced the original sources. It contains neither material previously published by another person nor material which has been accepted for the award of any other degree of the University.

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Table of Contents

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ABBREVATION AND ACRONYMS

ACN	Acetonitrile
ADLI	Agricultural Development Led Industrialization
AFB1	Aflatoxin Blue 1
AFB2	Aflatoxin Blue 2
AFG1	Aflatoxin Green 1
AFG2	Aflatoxin Green 2
AFs	Aflatoxins
DAD	Diode Array Detector
EFDA	Ethiopian Food and Drug Authority
ESA	Ethiopian Standards Agency
EU	European Union
FLD	Fluorescence Detector
GC	Gas Chromatography
HHICES	House Hold Income, Consumption and Expenditure Survey
H2O	Water
HPLC	High Presses Liquid Chromatography
MLs	Maximum Levels
NaCl	Sodium Chloride
ppb	Parts Per Billion
TLC	Thin Layer Chromatography
USA	United States of America
UV	Ultra Violet
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Red pepper is one of the most widely distributed food plants in the world and its infection by fungi can result mycotoxin contamination during the growing, harvesting, storage, transporting and processing stages It is widely produced in Ethiopia and is even regarded a national spice. However, aflatoxins can degrade this cereal crop, resulting in negative health effects in humans and cattle who consume aflatoxin-contaminated food. Both locally consumed and export red peppers were collected from the market and exporters site respectively. Using the HPLC technique with a method of AOAC Official Method 2005.08, this study was conducted experimentally to quantify the degree of Aflatoxin (AFB1, AFB2, AFG1 and AFG2) contamination in both domestically eaten and exported standard red pepper samples acquired from the Addis Ababa Administration. The calibration curve of standard solution aflatoxins in the range of 5-100ppb showed good linearity, with regression coefficient (R2) values of virtually > 0.998.The results of the study indicates that the total and individual aflatoxin concentrations are high in both locally consumed and export red peppers. The average results also show that locally consumed red pepper samples were more infected than export red pepper samples. The contamination of red peppers by aflatoxins need to be controlled by regulations to protect consumers.

Keywords: Aflatoxins, Mycotoxins, Red pepper, HPLC

CHAPTER ONE

INTRODUCTION

This chapter deals with background of the study, statement of the problem, research questions and objectives, theoretical framework/conceptual framework, significance of the study, limitation and delimitations of the study, definition of basic terms used in the study and finally organization of the study.

1.1. Background of the Study

Pepper (Capsicum annum L.) is an exotic crop for Ethiopia and believed to be introduced by the Portuguese in the 17th century and currently considered as the national spice. It is widely cultivated in different regions of Ethiopia specifically, Amhara, Oromia, and Southern Nation Nationalities and Peoples Regional State. Pepper is an important agricultural crop because of its economic importance, nutritional and medicinal value of its fruit. It is an important source of nutrients like vitamins A and C content; high iron, potassium, and magnesium provided in the human diet and it can be consumed fresh or dried. The range of food products that contain pepper or its chemical constituent is broad and includes ethnic foods, meat, salad dressings, mayonnaise, dairy products, and candies, packed foods, snack foods, salsa, and hot sauces (Tsehanesh. et al, 2021).

In addition to local consumption the world market need for spices is growing rapidly and the opportunity for Ethiopia to tap the market potential is huge. Among many problems affecting the development of the spice sector is the difficulty faced by the spice farmers to enter in to the world market which is dictated by global competition. The production of quality spices based on the needs of the international market is the necessary condition for export. A key requirement in service provision is timeliness, adequacy and ease of access. Defaults in delivery or quality are not accepted in the export environment. Many countries have established regulations to limit exposure to aflatoxin, typically expressed in parts per billion (ppb) (Abt Associates, 2012).

The word “mycotoxin” is derived from two words; “mukes” refering to “fungi” (Greek) and “toxicum” referring to “poison” (Latin).The word “Aflatoxin” is the combination of three words; “a” for the Aspergillus genus and “fla” for the species flavus, toxin for poison. Aflatoxin species have polycyclic structure; G series Aflatoxins are six-member lactones and B series Aflatoxins are pentanone derivatives (Bakirdere S. et al, 2012).

The most important fungi able to produce toxin are Aspergillus, Fusarium and Penicillium species. From various kinds of toxins the most important which are produced by these fungi are aflatoxins, ochratoxins, zearalinon and others. Those toxins are highly toxic compounds that cause many kinds of diseases including cancers. Although fungi growth requires moisture, they have the capability to grow on all foods without an exception, whether their moisture content was high or low. Fungi grow on crops in the field, during harvest, after crop harvest and storage (Essawet N. et al, 2017).

Fungi also grow within wide range of temperatures (15- 35 ºC) and cause the damage of physical (in shape, texture, color, aroma and taste) and chemical (due to the fungal consumption of nutrients so lowering the food content of organic matter) changes of foods in addition to the aflatoxins that they secret. It is not necessarily means that every fungi infected commodity is containing aflatoxins because fungal growth needs conditions different from that needed to produce toxins such as the moisture of the infected commodity, medium temperature, medium content of oxygen and other required conditions for fungi to grow and produce toxins. However, not every fungus has the genetic capacity to produce aflatoxins even he belongs to one species known with aflatoxins production due to the differences between the isolated strains from the same species which are accompanied with differences in the capability of toxin(s) production according to the genetic capacity (Essawet N. et al, 2017).

Tropical climates with high temperature and humidity are suitable conditions for mycotoxin contamination and spices are mostly produced in countries with tropical climates. Spices are exposed to a wide range of microbial contamination as a result of improper production process, extended drying times and poor storage conditions and red pepper flakes is a very sensitive product

for aflatoxin formation depending on unsuitable processing conditions because they are usually dried on the ground in the open air (Gulderen Y. et al, 2012). The hot and humid climate, production conditions with extended drying times, and often-inadequate instructions to the farmers, may cause considerable quality problems (Aydin A. et al, 2007).

The drying and storage phases are critical in the pepper production chain. Traditionally, peppers are dried by direct sun exposure or mechanical heat drying; these methods take extended periods of time at different temperatures and humidity or at higher controlled temperatures for short time periods, respectively. The whole pepper production chain should be managed carefully to prevent fungal infection and mycotoxin contamination (Costa J. et al, 2019).

Mycotoxins are fungal secondary metabolites mostly produced by toxigenic molds. AFs are a major group of mycotoxins, mainly produced by the species of Aspergillus, specifically Aspergillus flavus and Aspergillus parasiticus. AFB1, AFB2, AFG1, and AFG2 are the most mycotoxins that appear in foodstuffs as a result of fungal contamination during harvest and post-harvest practices (Barani A. et al, 2016). And among all mycotoxins, AFB1 is considered to be the most carcinogenic (Chauhan, N.M. et al, 2016).

Aflatoxins, each of which is a group of closely related mycotoxins, may be produced by three of Aspergillus species (i.e.A. flavus, A. parasiticus, and the rare A. nomius) which contaminate plants and plant products. Aspergillus flavus produces merely the B type of Aflatoxin, while the other two species produce both Aflatoxins B (B1, B2), G (G1, G2) and the term "B" and "G" refers to the colors blue and green products fluorescence UV light on TLC plates; while the number 1 and 2 show the respective major and minor compounds (Ketney O. et al, 2014). Ethiopia is among the few developing countries that have been producing paprika and Capsicum oleoresins for the export market. Apart from its food importance, pepper is one of the most important spices that serve as the cash crop and source of income for smallholder producers in many parts of rural Ethiopia (Tsehanesh. et al, 2021).

Red pepper is the most important world spice crop and the second largest consumed spice throughout the world. In Ethiopia, pepper is the most dominantly grown spice and is a high value crop for household consumption and for sale both at domestic and export markets playing an important role in the national economy. All analytical methods for aflatoxin analysis involve basically the same steps: sampling and sample extraction, cleanup, work-up detection and confirmation, as well as estimation of the toxin. Currently aflatoxin analysis are done by various methods including thin-layer chromatography (TLC), gas chromatography(GC), high-performance liquid chromatography (HPLC), Immunochemical methods and enzyme-linked immunosorbent assay (Cornett A. et al, 2012).

About 80% of organic compounds in the world are determined using HPLC. The HPLC technique makes use of a stationary phase confined to either glass or plastic tube and a mobile phase comprising aqueous/organic solvents, which flow through the solid adsorbent (Aiko V. & Mehta A. 2015). Aflatoxins are generally soluble in organic solvents as methanol, acetone, chloroform and acetonitrile. Thus, for their extraction these solvents or mixtures are used. Extraction varies according to the degree of selectivity, rapidness, convenience, and depends not only on access and conditions at which it is carried out, but also on the configuration of extraction stages (Georgievski B. et al, 2016).Therefore, the aim of this research is to determine the level of Aflatoxins in red pepper ready for local consumption and export purpose in Addis Ababa by using HPLC method which is preferred as better aflatoxins determination technique than other techniques described above.

1.2. Statement of the Problem

Aflatoxins are mycotoxins with high toxicity and occurrence which affects the human health and trade (Gnonlonfin G. et al, 2013). Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade. Aflatoxin contamination is a serious food safety problem throughout the world and in Ethiopia it is much more serious due to traditional practices of pepper production process (Habtamu. et al, 2001). Mycotoxin contamination of human food and livestock feeds has come to the attention of the WHO due to global concern in relation to human and animal health. Aflatoxins

are the most potent naturally occurring carcinogenic as well as immunosuppressive (Geremew, 2015). In Ethiopia the problem of aflatoxin contamination in agricultural commodities is much more serious. Red pepper is highly affected by mycotoxin which is hazardous to human when consumed as food (Abbas H.K. & Wilkinson J.R.Z.R.M., 2009).

Studies are conducted in the world to determine the Aflatoxin content of red peppers for consumption. AFB1 contamination of red pepper has also been reported from Ethiopia, where samples collected from markets, shops and storage facilities were contaminated with AFB1 in concentrations of 250-525 ppb (Essawet N. et al, 2017).
Additionally, Studies indicate that AFB1 contamination of red pepper in the range of 1.25 - 28.50 ppb, 8 - 35 ppb and 100 - 500 ppb in different samples. In India AFB1 was determined and 59% of the collected red pepper samples from the markets were contaminated with AFB1, and the AFB1 level in 18% of them was above the accepted limits, 30 ppb. In a market survey conducted by the Ethiopian Ministry of Agriculture, it was stated that 46.7% of powdered peppers contain more than 5.0 ppb of AFB1 and that peppers are the most contaminated product in terms of AFB1 (Kiric A., 2019).

Red pepper post-harvest storage practices including the drying culture are not good in Ethiopia. Due to these causes the safety of red pepper is at risk for being contaminated by aflatoxins. If red pepper becomes contaminated by aflatoxins in these ways, consumers become affected due to the carcinogenic behavior of aflatoxins. Similarly there are several exporters in Ethiopia engaged in the export of paprika powder to countries abroad. There have been incidents where exported paprika powder contained mycotoxins exceeding the regulatory limit. When exported red peppers are found to contain levels of mycotoxins exceeding the regulatory limit, the exported food item is usually quarantined and destroyed. In addition to the financial losses incurred, these incidences damage the country's reputation in the international trade. And since Ethiopia is a country that relies on its export for acquiring access to foreign currency, rejected exports will have a strong impact on the country's financial wellbeing (Habtamu. et al, 2001).

All these aspects combined make the risk of exposure to these contaminants worth investigating. Although, some studies analyze the contamination of red pepper by aflatoxins no one of the previous researchers evaluate the basic groups of aflatoxins (AFG2, AFG1, AFB2 and AFB1). They only determine AFB1 content by ignoring the three aflatoxin types (AFG2, AFG1 & AFB2).However; in addition of AFB1, the ignored aflatoxin types (AFG2, AFG1 & AFB2) were determined in this research. The contamination amount of all those aflatoxin types was also compared in locally consumed and exported red pepper products. Therefore, the aim of this study was to determine the aflatoxins contamination of red pepper in the practice of current production process and ready for local consumption or export purpose in Addis Ababa city by using high performance liquid chromatography (HPLC) technique.

1.3. Research Questions

After completing this research study, the following questions were answered based on the results of completed research.

- What is the extent of aflatoxins (AFB1, AFB2, AFG1, and AFG2) in red pepper ready for local consumption in Addis Ababa?
- What is the extent of aflatoxins (AFB1, AFB2, AFG1, and AFG2) in red pepper ready for export purpose in Addis Ababa?
- From red peppers ready for local consumption and export which product is more contaminated by aflatoxins?

1.4. Objectives of the Study

1.4.1. General Objective

- The aim of the proposed study was to determine the amount of Aflatoxins in red pepper ready for local consumption and export purpose in Addis Ababa by using HPLC technique.

1.4.2. Specific Objectives

- To determine the extent of aflatoxins (AFB1, AFB2, AFG1, AFG2) contamination in red pepper ready for local consumption.

- To determine the extent of aflatoxins (AFB1, AFB2, AFG1, AFG2) contamination in red pepper ready for export purpose.
- To compare the contamination of aflatoxins in red pepper ready for local consumption and export.

1.5. Conceptual Framework

Mycotoxins are fungal secondary metabolites mostly produced by toxigenic molds. Aflatoxins (AFs) are a major group of mycotoxins, mainly produced by the species of Aspergillus. These toxins are highly toxic compounds that cause many kinds of diseases including cancers. They also attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade.

Red pepper is the most important world spice crop and the second largest consumed spice throughout the world. In Ethiopia, pepper is the most dominantly grown spice and is a high value crop for household consumption and for sale both at domestic and export markets playing an important role in the national economy. However, the process of pepper production in Ethiopia have several problems like storage practices before and after processing, lack of awareness of producers and traders about factors affecting the quality of red pepper, addition of water to the red pepper in the market chain to increase weight and color which intern increases the contamination level of the product by mycotoxins. Red pepper is one of the products sensitive to AFs formation due to the conditions it faces during production, harvest, drying and further processing.

Red pepper can be protected from mycotoxins by controlling the fungi developing factors like moisture, humidity and temperature. To know the effect of these practices on the quality of red pepper it is essential to investigate the aflatoxin levels present in this product both in locally consumed and exported. High performance liquid chromatography with a method of internationally accepted AOAC Official Method 2005.08 is preferred to determine the aflatoxins contamination levels in foods. Many studies are indicating that aflatoxins can exist in various concentrations in red peppers as a result of the growth of the productive fungi and the availability of suitable moisture and temperature for fungal growth.

1.6. Significance of the Study

The finding of this study is helpful to know the amount of aflatoxin contamination in red pepper and also important in keeping public health of the people by increasing knowledge and awareness of aflatoxin contamination on agricultural food products. The study on the status of aflatoxin amounts in commercially available red pepper and ready for export in Addis Ababa is significant at this time to understand the current situation in the particular study area.

Therefore, it will help in creating awareness to manufacturers, exporters and consumers on red pepper about its aflatoxin contamination. It will also assist regulatory bodies like Ethiopian Standards Agency (ESA), Ethiopian Food and Drug Authority (EFDA) and Ethiopian Ministry of Trade in implementing regulations on aflatoxin levels for red pepper. Moreover the study will provide a base line data for the next researchers.

1.7. Delimitations of the Study

In this research the contamination amounts of aflatoxins (AFG2, AFG1, AFB2 and AFB1) which are toxic and carcinogenic for human health were determined from different red pepper samples being ready for local consumption and export. Red pepper samples which are ready for export were collected from exporters processing site in Addis Ababa city. Similarly samples that are prepared for local consumption were also collected from different shops found in Addis Ababa city. AOAC Official Method 2005.08 was used to determine the level of all aflatoxins from these samples by using the instrument called HPLC.

1.8. Limitation of the Study

This research study includes red pepper samples which are ready for export and needs direct access of exporter's site. At this time access of exporters' site was challenging for some exporters due to their voluntariness to deliver the samples.

1.9. Definition of Basic Terms Used in the Study

Spice: refers to any dried plant product used primarily for seasoning, be it the seed, leaves, bark/peel, or flowers. Spices are essential oils that give foods and beverages flavor, aroma, and sometimes color (Wubalem, 2019).

Chromatography: is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis (Coskun D.O, 2016).

Mycotoxins: are toxic metabolic substances formed due to improper feed storage and may result in massive outbreak of diseases in humans and animals (Rasha M.E & Amal A., 2020).

Aflatoxins: are groups of mycotoxins with high toxicity and occurrence which affects the human health (Gnonlonfin G et al, 2013).

Evaluation: is the systematic assessment of the design, implementation or results of an initiative for the purposes of learning or decision-making (Canadian Evaluation Society, 2015, October).

1.10. Organization of the Study

The research will be presented in five chapters. The chapters are Introduction, Review of Related Literatures, Research Design and Methodology, Results and Discussion and finally Summary, Conclusion and Recommendation respectively. In each chapter different ideas will be discussed. Chapter one covers background of the study, statement of the problem, research questions, research objectives, Conceptual Framework, significance of the study, limitations and delimitations of the study and definition of basic terms. Chapter two comprises the literatures on related researches. Chapter three explains the research design, the research method and research materials and method will be presented. In chapter four research results and discussions on the results will be included. Finally in chapter five research summary, conclusion and recommendations will be presented.

CHAPTER TWO

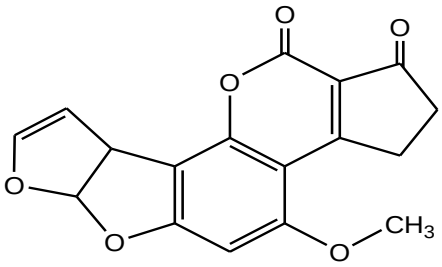
REVIEW OF THE RELATED LITERATURE

This chapter presents literatures relevant to this study having related scope and concept. Different supportive research outputs with related scope will be assessed and organized in this chapter. These outputs of related researches will be used as benchmarks for this study.

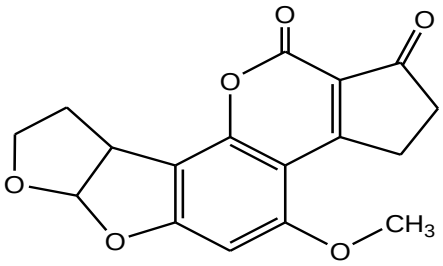
2.1. Structure, Nomenclature and Occurrence of Aflatoxin B&G

The four major naturally occurring aflatoxins are aflatoxins B1, B2, G1 and G2. Aflatoxin B1, B2, G1 and G2 belong to a group of around 20 similar chemicals. The letters 'B' and 'G' stand for the blue and green fluorescent colors produced by these compounds when exposed to UV light, respectively, while '1' and '2' stand for the major and minor components. The dihydro derivatives of the main ('1') metabolites make up the '2' chemicals. Only 'B' aflatoxins are produced by A. flavus (AFB1 and AFB2). Both 'B' (AFB1 and AFB2) and 'G' (AFG1 and AFG2) aflatoxins are produced by A. parasiticus. A. nomius' status looks to be comparable to that of A. parasiticus. Aflatoxin B1 is the most frequent aflatoxin in foods, and it's also the one that's been examined the most in toxicological studies. A. flavus is widespread in the environment, but A. parasiticus is much less so (Cressey P. et al, 2009).

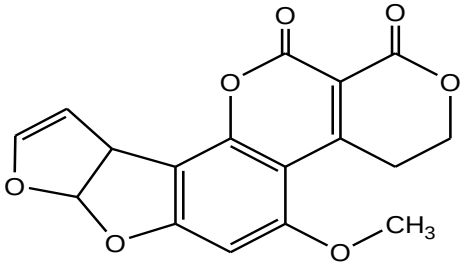
Figure1. Chemical Structures of Aflatoxins B&G



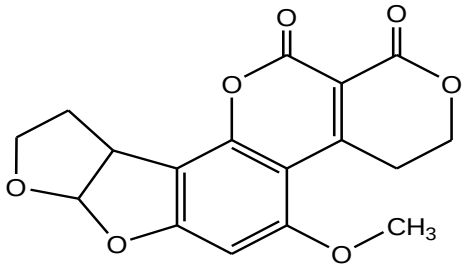
Aflatoxine B1



Aflatoxine B2



Aflatoxine G1



Aflatoxine G2

2.2. Food Safety and Quality

Food safety pertains to human health and is defined as the absence of toxins or allergens in food, or their presence below consumer-safety criteria. Food quality refers to a product's capacity to meet users' declared or implied needs. Simply put, quality can be defined as suitability for a specific purpose. In the case of food, safety, nutritious value, and acceptance are the most important factors to consider. Food safety must be included in policy when determining whether or not a product is safe to consume. Poison-free food ensures that consumers are protected from infection (Buckwell A. 2015).

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As a result, policies must be applied as rules in order to control food quality. Food processing and production, transportation, storage, distribution, nutrition and health principles, and points of import and export are all examples of regulations. Food safety is a significant area of market failure, necessitating a highly established network of EU, regional, and local regulatory mechanisms. The manufacture of consistently high-quality products necessitates a tremendous deal of attention, promotion, and financial investment, as well as a great degree of risk.

Maintaining food safety and quality boosts market share while also safeguarding consumers' health. Quality control refers to the procedures that ensure that food items meet customer expectations in terms of physical, chemical, microbiological, and nutritional value (Buckwell A. 2015).

2.3. Red Pepper and Mycotoxins

Pepper is the most frequent and widely consumed item in practically every Ethiopian's daily diet. Pepper is consumed to boost meal intake as well as to supplement dietary demands in the home. The majority of smallholder growers in the south, Oromia, and Amhara regions grow red pepper as a main spice. Despite the importance of red pepper in Ethiopia's economy and current income-generating capacity, it has received little attention in the country (Wubalem, 2019).

Mycotoxins can be present in red pepper during the pre-harvest, postharvest, and storage stages. According to reports, fungi have infected agricultural products around the world to the point where they can no longer be ingested by humans or animals. Spices are primarily produced in nations with good environmental circumstances, such as a wide temperature, humidity, and rainfall range. During the manufacture of red peppers, mycotoxins might develop due to incorrect storage, long drying durations, and excessive moisture content. Flatoxins are produced by the fungi Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius, and are the most prevalent mycotoxins found in spices. These mycotoxins are carcinogenic to people and animals, resulting in a variety of health issues. Aflatoxin is the most poisonous and carcinogenic member of this family (Tosun1 H. & Arslan R., 2013).

Fungi have the ability to create and infect AFs found in the flora of agricultural products and various foodstuffs. The production of AFs, as well as degradation, occurs when favorable conditions are formed for their development. Due to the conditions it encounters during cultivation, harvest, drying, and further processing, red pepper is one of the goods susceptible to the creation of AFs (Kiric A., 2019).

2.4. Red Pepper Processing and Storage Practices in Ethiopia

Pepper thrives in Ethiopia's hot, humid climate, with the best fruit produced at temperatures of 21-27 degrees Celsius during the day and 15-20 degrees Celsius at night (Seleshi, 2011). Ethiopian smallholder farmers generate the majority of red pepper. The production of red pepper, on the other hand, was influenced by a variety of institutional, environmental, and social factors. In most production locations, the main red pepper production restrictions include a lack of agricultural inputs, the presence of red pepper disease, the lack of new varieties and low productivities of native varieties, and a lack of land. Furthermore, red pepper production is labor-intensive by nature (Abebe et al, 2019) and inadequate storage practices as one of the affecting pepper production process to be challenging and producers face storage loss and quality deterioration. To solve these problems constructing storage and processing facilities would be very important. Storage facilities are very important for marketing to make red pepper available at required season (Tesfasilassie et al, 2017).

Water activity is the most critical component for fungus growth and toxin production. By removing the water in the product, all microorganisms, including fungus, are stopped from growing. To minimize humidity, pepper is frequently dried in the sun. Drying should be done in a way that does not compromise the product's quality. Fungi commonly grow from air and ground during traditional drying (in the sun). Due to poor drying and storage conditions, they may occur during or after harvesting. Drought and insect damage to agricultural goods can raise the level of fungal contamination (Ozturkoglu-Budaki S., 2017).

2.5. Related Studies of Aflatoxins

As a result of the establishment of productive fungi and the availability of optimal moisture and temperature for fungal growth, several studies show that aflatoxins can occur in varied amounts in various types of spice samples. Aflatoxin contamination affects peppers in a variety of ways, including temperature, humidity, insects, and drying and processing conditions. When the conditions are right, mold contamination can happen during the agricultural production process and during storage (Essawet N. et al, 2017).

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Sun drying, which involves spreading peppers on soil, is a typical postharvest procedure in various areas. The conditions that cause peppers to become fungus-infected are influenced by soil drying processes. AFB1 contamination of ground red pepper has been recorded in Ethiopia, where samples taken from marketplaces, stores, and storage facilities were contaminated with AFB1 in amounts ranging from 250 to 525 grams per kilogram. (Essawet N. et al, 2017).

Various research have been carried all over the world to assess the Aflatoxins concentration of red peppers for human consumption. Since there have been returns of exported peppers from European countries, the presence of AFs in red pepper has gotten a lot of attention. AFB1 contamination levels in different spice samples ranged from 8 to 35 ppb, according to studies. Red pepper samples obtained from Ethiopian markets, on the other hand, exhibited AFB1 levels in the range of 100-500 ppb, according to studies. AFB1 was found in a large percentage of red pepper samples gathered from Indian marketplaces, and some of the AFB1 levels were found to be beyond the permitted limits (30 ppb). According to Ethiopian Ministry of Agriculture market analysis, 46.7 percent of powdered and red chili peppers have more than 5.0 ppb of AFB1, making peppers the most contaminated commodity in terms of AFB1 (Kiric A., 2019).

2.6. Regulations of Mycotoxins

Some countries have different limits depending on the intended use, the tightest applying to human consumption and exports, and the highest to industrial products. Specifically, in the international market, products that do not meet the aflatoxin standards are either rejected at the border, rejected in channels of distribution, assigned a reduced price, or diverted to non-human or even non-fee uses. Similar economic losses may occur in domestic markets if consumer awareness about the problem rises, if leaders in marketing channels begin to pay more attention, and/or if regulations are either tightened or more strictly enforced (Abt Associates, 2012).

Regulatory bodies should support and ensure efficient and orderly management of the red pepper value chain. Specifications must be in line with the Ethiopian standards agency (ESA) format and must contain analysis results produced by a qualified lab and applying ESA certified procedures as indicated in the requirements of the spices in the European Union (EU) and the USA market (Solomon D. et al, 2010 February). However, red pepper production processes for both local consumption and export purposes have been facing food safety challenges from contamination of mycotoxins. And red pepper is vulnerable to mycotoxin contamination along the production chains by Aspergillus species (Tariku et al, 2020).

Through food, water, air, and direct touch with the body, humans are exposed to a vast spectrum of chemicals. In this regard, mycotoxins are among the most carcinogenic natural and unavoidable chemical pollutants that humans are exposed to on a daily basis. Fungal deterioration and mycotoxin contamination of foods are global issues that cost billions of dollars and pose a severe threat to human health. Several research on mycotoxins contamination in foodstuffs, including cereals, nuts, and spices, have been published in various countries, particularly in Asia and Africa. To protect consumers from the hazardous consequences of mycotoxins, numerous countries have enacted legislation governing these substances (Yogendrarajah P., 2015).

Because it is impossible to completely remove food contamination, maximum levels (MLs) should be set at a stringent level that can be achieved by using excellent agricultural and manufacturing methods and considering the danger of food intake. When defining regulatory sampling criteria, the distribution of mycotoxins in products is a crucial factor to consider. In several nations, mycotoxin rules have been developed, and new restrictions are continually being published (Yogendrarajah P., 2015).

The Codex Alimentarius Commission establishes international food and feed legislation (CAC). The Codex Alimentarius system is established for the creation of legislation concerning pollutants in foods and feeds, including mycotoxins. Mycotoxin rules have become more harmonized between nations that belong to the same economic communities since the formation of large economic communities. The EU has the most thorough food mycotoxins legislation in the world.

In spices, the most common standard for AFB1 is 5 g/kg, while the most common limit for the combined AFB1, B2, G1 and G2 is 10 g/kg (Yogendrarajah P., 2015). The Ethiopian Standards Agency (ESA) likewise recognises and approves the Codex of the European Commission Regulation on aflatoxins limits, and has designated it as an ES ISO standard (ESA, 2012).

2.7. Market Profile of Red Pepper in Ethiopia

Ethiopia is pursuing a market-oriented economic policy and development strategy that is centered on agricultural growth and industrialization (ADLI). According to the Household Income, Consumption, and Expenditure Survey -HHICES, spices account for 1.79 percent of total household expenditure, with red pepper accounting for the largest share, followed by ginger, fenugreek, and cinnamon. Ethiopian pepper is imported by the United States, Israel, Australia, Canada, and Djibouti, with the United States being the biggest importer (Masresha, 2010, November).

2.7.1. Major Constraints in Red Pepper production and marketing

Production, gathering, drying, storage and processing, transportation, and marketing are all part of the red pepper market chain. The pepper sector in Ethiopia is plagued by numerous issues, the most serious of which occur throughout the manufacturing, processing, and marketing stages. The role of private business investors in regulating the quality of red pepper manufacturing is limited, and as a result, low-grade products are widely distributed. Wastage/spillage and product quality deterioration are caused by improper production methods, particularly post-harvest handling techniques and marketing systems (Masresha, 2010, November).

Weak business links among players in the production and market chain, such as farmers, traders, processors, and support organizations such as regulatory and enforcement authorities, also impair red pepper quality and safety. The pepper is at risk of deteriorating due to a lack of proper drying, transportation, and storage management. Keeping the product in the store for a long time in the hope of higher prices, trading of poor quality red pepper due to highly traditional pre and post-harvest handling practices, adding water to increase weight and also color/appearance, and poor

market research and promotion in potential foreign markets are all problems in the marketing stage (Masresha, 2010, November).

2.8. Conditions of Fungal Growth and Mycotoxin Production

Mycotoxigenic fungi are prevalent diseases found throughout the world's agricultural regions. Fungal growth and mycotoxin production are affected by a variety of factors, and contamination with these toxins can occur at various points along the food chain because it is an accumulative process that can begin in the field and increase during later stages such as harvesting, drying, and storage. There are two types of fungal growth: primary and secondary. While primary growth necessitates the use of organic compounds for biomass synthesis and energy production to drive chemical reactions and produce primary metabolites required for growth, secondary growth occurs after a period of stable growth and may, but not always, result in sporulation and the production of secondary metabolites such as mycotoxins (Daou R. et al, 2021).

Temperature, water activity, relative humidity, and pH are all important factors in mycotoxin formation. Climate elements have a crucial role in influencing fungal occurrence; therefore the fungi's activity and amount of colonization are heavily influenced by prevalent environmental conditions, particularly humidity and temperature. For development, germination, and mycotoxin generation, each fungus has an ideal temperature and water activity range. Temperatures between 25 and 30 °C, a water activity more than 0.78, and relative humidity between 88 and 95 percent are all regarded suitable for fungal development and the generation of mycotoxin. The PH value, or hydrogen atom saturation in the media around the fungus, influences its growth either directly on cell surfaces or indirectly on food availability. In most cases, acidic circumstances encourage germination and the development of mycotoxin. A pH of 4.0 is required for the creation of aflatoxin, and the lower the pH, the higher the synthesis (Jallow A. et al, 2021).

2.9. Mycotoxins Control and Prevention Strategies

Because the creation of mycotoxins is inescapable in nature, most foods are at danger of contamination. There is currently no mechanism for 100% control of mycotoxins, and developing a food safety policy for contamination control is difficult. Adopting an integrated food safety system that includes good quality procedures at each stage of production to reduce the frequency of mycotoxins in the final product could be a beneficial solution. Applying proper precautions during pre-harvest, harvest, drying, storage, and processing are examples of such activities (Daou R. et al, 2021).

Contamination control during the pre-harvest period is critical since it is the initial point of entry for mycotoxin into food. Harvest is a vital step for mycotoxin control, with moisture content becoming the most significant criterion for mycotoxin control. When deciding when to harvest, three variables must be considered: the main weather conditions, the risk of bug, pest, rodent, and bird infestation, and the availability of drying facilities and storage warehouses. Harvesting should begin after a period of dry weather under ideal circumstances. It's also critical to inspect the cleanliness and hygiene of the harvesting equipment to ensure that no fungal cross-contamination occurs from one batch to the next. Because of the high moisture content of the harvested product, it must be dried properly before being stored safely (Tumukunde E. et al, 2020).

To avoid fungal assault, it is critical to achieve the proper moisture content during drying. Storage is another key stage in the food chain that might affect later product safety and quality, so it's critical to keep storage conditions under rigorous supervision during the whole keeping period. Storage fungus can cause significant damage, including the development of mycotoxin, a decline in quality, and nutritional losses (Tumukunde E. et al, 2020).

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

This chapter contains information about the research design, sampling and sample handling, the research method, materials and sample preparation, extraction, clean-up and determination of aflatoxins.

3.1. Research Approach

A cross-sectional study design was used for the determination of aflatoxins from red pepper samples which were ready for local consumption and export purpose separately (Setia S.M.Dr et al, 2016). I have used this study design because samples were directly collected from market and the investigation was done experimentally.All analytical laboratory works were carried out at Bless Agri Food Laboratory Services PLC, the largest private food and agricultural testing laboratory in Ethiopia located in Lege Tafo, Oromia.

3.2. The Research Method

HPLC methods had been developed to determine aflatoxins from agricultural commodities. Determining aflatoxins by those methods is after extraction, partitioning and derivatization of food samples. In HPLC a liquid mobile phase or solvent was used to move the sample through the column. An immobilized solid stationary phase was packed in the column. The analyts were then partitioned between the two phases as they pass through the column and thus leading to the separation of compounds due to the difference in partitioning coefficients.

Both normal phase HPLC (a polar stationary phase and a non-polar solvent) and reverse-phase HPLC (non-polar stationary phase hydrocarbons and polar mobile phase) separations had been developed for aflatoxin analyses. Normal phase columns with different detectors like; ultra violet (UV) detector, diode array detector (DAD) or a fluorescence detector (FLD) were used to determine Aaflatoxin. Fluorescence detection and electrochemical detection were the two sensitive

detection methods most commonly used for quantitative studies in HPLC. FLD detection, Official Method of Analysis (AOAC 2005.08.), which is international method for the determination of aflatoxins, was used for this research study (William H. Dr. & George W.L.Jr., 2005).

3.3. Sample Size

The total numbers of samples analyzed for this study were 20. The number of samples to be 20 was decided by referencing other similar researches. From a total of 20 samples, 10 samples were from local market and 10 were from exporters' site (Essawet N. et al, 2017) & (Geremew. 2015).

3.4. Sampling and Sample Handling

A total of 10 red pepper samples were collected from Addis Ababa administrations that were ready for export from the site of different exporters. And also a total of 10 red pepper samples were collected from Addis Ababa administrations that were ready for domestic consumption from different shops. Samples were collected from shops and exporter's site via systematic random sampling method. The collected samples were transported to the laboratory and stored at room temperature until the time of analysis.

S.NO	Sample	Sample Type	Area of Sample Collection
1	LS01	Locally Consumed Red Pepper	Goro, Addis Ababa
2	LS02	Locally Consumed Red Pepper	Megenagna Shola, Addis Ababa
3	LS03	Locally Consumed Red Pepper	Kotebe 02, Addis Ababa
4	LS04	Locally Consumed Red Pepper	Mexico, Addis Ababa
5	LS05	Locally Consumed Red Pepper	Zenebwork, Addis Ababa
6	LS06	Locally Consumed Red Pepper	Garment, Addis Ababa
7	LS07	Locally Consumed Red Pepper	Ayertena, Addis Ababa
8	LS08	Locally Consumed Red Pepper	Addisu Gebeya, Addis Ababa
9	LS09	Locally Consumed Red Pepper	Merkato, Addis Ababa
10	LS10	Locally Consumed Red Pepper	Kotebe Kara, Addis Ababa

11	ES01-ES10	Export Standard Red Pepper	From Different Exporters Site, Addis Ababa

Table 1: Samples and Areas of Collection

3.5. Materials

All chemicals, apparatus and accessories used for analysis in the research have analytical grade and obtained from well-known company. Those chemicals, apparatus and accessories were used according to AOAC Official Method 2005.08. All those chemicals, apparatus and accessories were available at Bless Agri Food Laboratory with the access to use those for my research (William H. Dr. & George W.L.Jr., 2005).

HPLC system consisting of an auto sampler with injector, pump, column oven, and fluorescence detector; computer with chromatography software, UVE™ Photochemical Reactor, C-18 Reversed Phase HPLC Column, Immunoaffinity column, Sample reserviour (100ml for sample load, reusable, including screwing and seal, P/N 10896), Sample Grinder (Mixer),Filter Paper (90 and 150 mm), Measuring Cylinder, Vacuum Pump, Adjustable Pipettes, Micro filter, shaker(extractor), Analytical balance, Variable Volumetric flask, Glass separatory funnels (250 ml), Filtering flask, Funnel (90mm), Micro syringe, Pipet tips (100 and 1000 µl), Buchner funnel (90mm), Vials with screw and septum, Glove and beakers (100 and 250 ml) were used. The chemicals were Aflatoxin standards solutions (AFG1, AFG2, AFB1 and AFB2), Acetonitrile, Methanol, N-Hexane, Water, Buffer and Sodium chloride (William H. Dr. & George W.L.Jr., 2005).

3.6. Sample Preparation, Extraction, Clean-up and Determination

3.6.1. Sample Preparation

As the distribution of aflatoxin is extremely non-homogeneous, laboratory samples were grounded with grinding device and then homogenized before conducting laboratory tests. The grinder was

cleaned by acetone before and after grinding in order to prevent cross-contamination of aflatoxins. Aflatoxins were analyzed in red pepper according to the method described. First aflatoxin mix standard solutions (containing 5, 10, 20,30,40,50 and 100 ug/kg) were prepared in methanol from stock aflatoxin mix standard. Then HPLC was calibrated by those standard solutions (William H. Dr. & George W.L.Jr., 2005).

3.6.2. Extraction, Clean-up and Determination

20 g of prepared sample with 2.0 g of NaCl was added to extraction solvent mixture of 100 mL (methanol: water (80:20 v/v)) in a beaker and was shake for 50 minutes in shaker at high speed. The extract was filtered through what man filter paper No.4. Then, 7 mL of the filtered extract was taken and diluted to 50 mL with PBS, and 50 mL of the diluted filtrate was applied to the immune affinity column previously conditioned with 10 mL of PBS (flow rate of about 2 ml/min). The column was washed with 10 mL of water and air was drawn through the column until dry. Aflatoxins were eluted by applying 2 mL of methanol to the column and the elate was injected on to the HPLC system. Extraction variables were controlled by running reference sample having known value of aflatoxins.Aflatoxins were determined by injecting 20ul of elate in to the HPLC system with the mobile phase of Water/Methanol/Acetonitrile (60/25/15, v/v) (William H. Dr. & George W.L.Jr., 2005).

CHAPTER FOUR

RESULTS AND DISCUSION

4.1. Instrument Calibration Curve

Fluorescence detector HPLC system was calibrated by injecting different concentrations of the four aflatoxins covering the concentration ranges from 0.5 to 10µg/L for Aflatoxin G2; 2 to 40µg/L for Aflatoxin G1; 0.5 to 10µg/L for Aflatoxin B2 and 2 to 40µg/L for Aflatoxin B1 in a mobile phase of ACN: H2O: MeOH for linearity. The elution order of individual aflatoxin based on polarity was in the order of AFG2, AFG1, AGB2, and AFB1 with approximate retention tome of 5, 6, 7 and 8 respectively. Peak areas of the different aflatoxins were plotted against the concentrations and linear regression analysis was used to calculate the equation and the correlation coefficient of the standard curves. All correlation curves were with goodness of fit (R^2) >0.998, demonstrating the linearity over the concentration ranges studied.

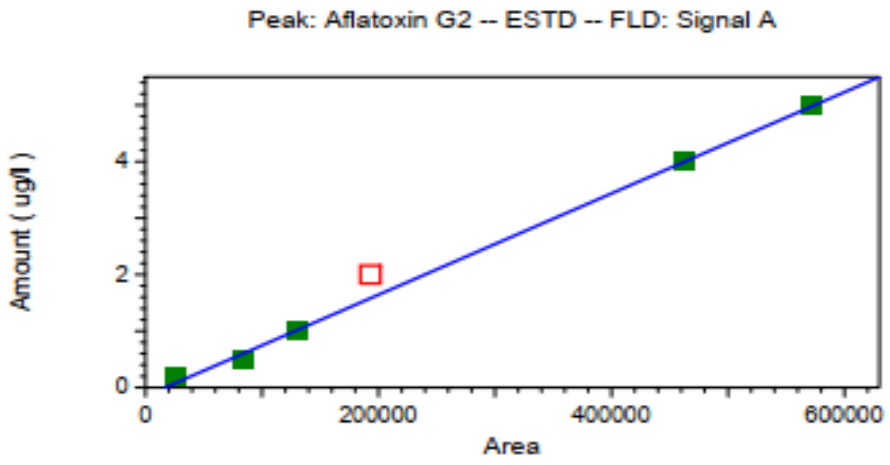


Figure 2: Instrument Calibration Curve for Aflatoxin G2 by using serious of standard solutions

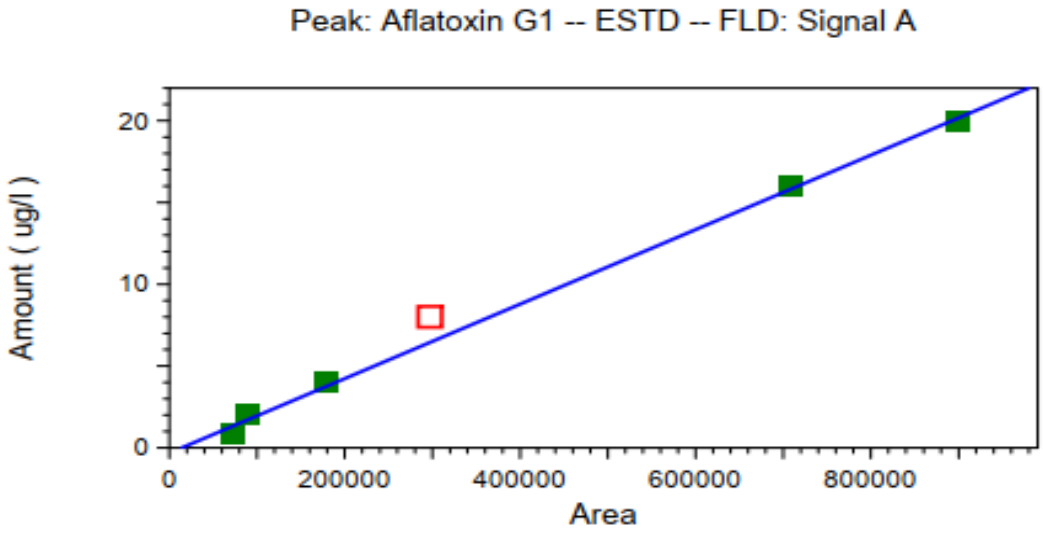


Figure 3: Instrument Calibration Curve for Aflatoxin G1 by using serious of standard solutions

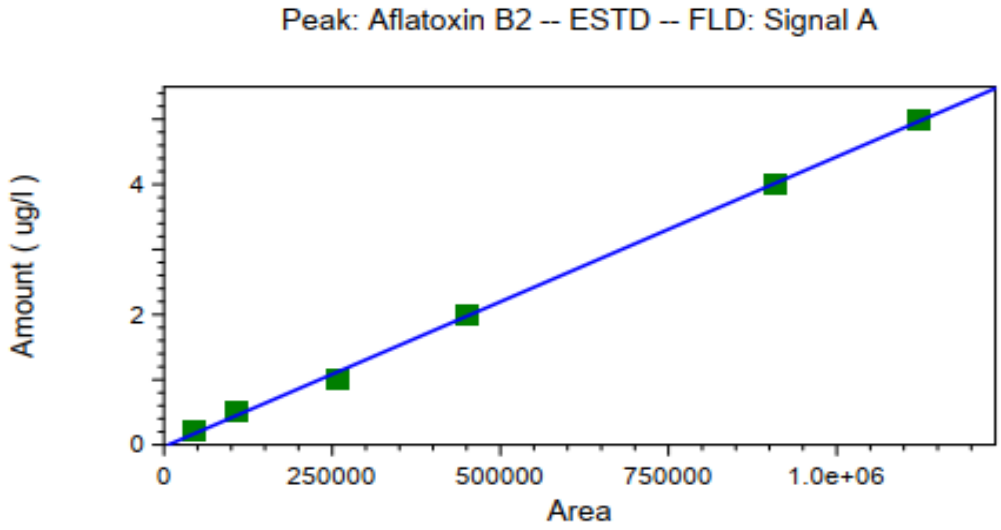


Figure 4: Instrument Calibration Curve for Aflatoxin B2 by using serious of standard solutions

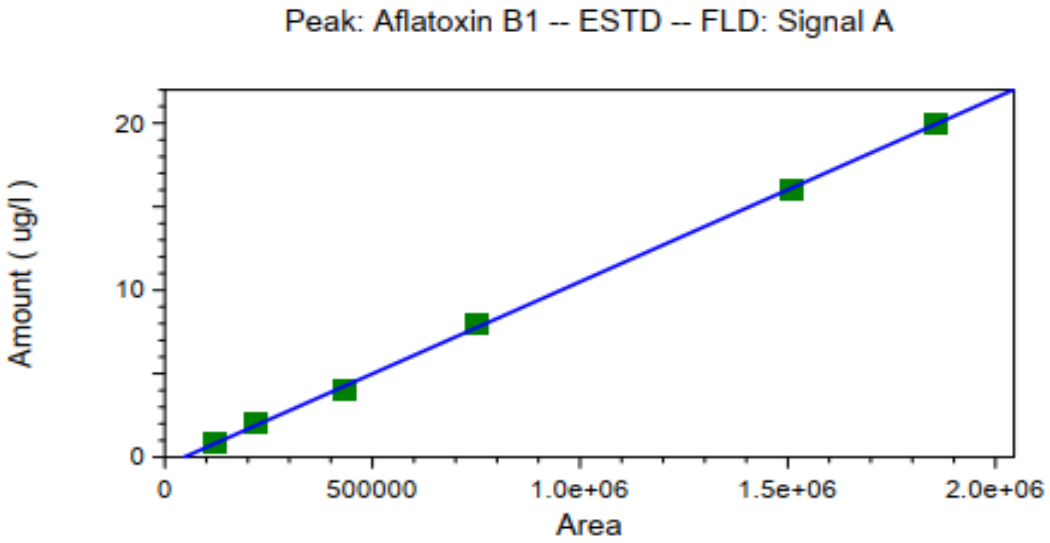
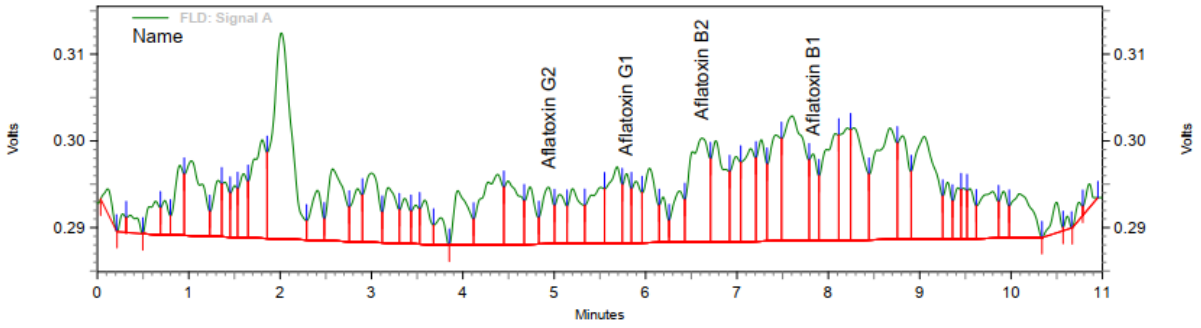


Figure 5: Instrument Calibration Curve for Aflatoxin B1 by using serious of standard solutions

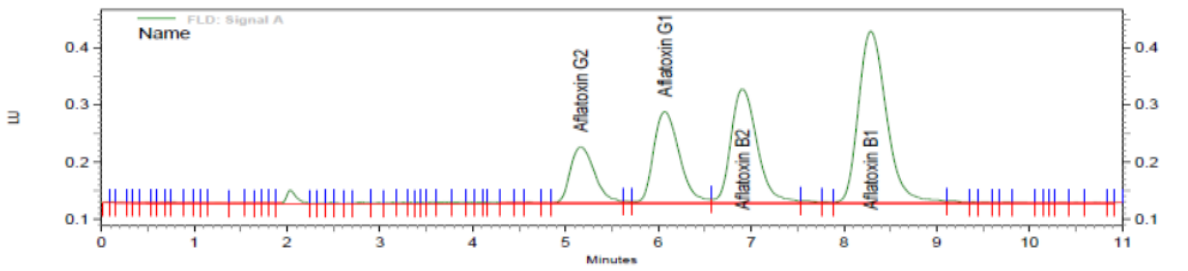


FLD: Signal A Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
27	Aflatoxin G2	4.939	9089	**0.000**
32	Aflatoxin G1	5.789	7334	**0.000**
37	Aflatoxin B2	6.617	28348	**0.096**
44	Aflatoxin B1	7.834	9898	**0.000**

Totals		54669	**0.096**

Figure 6. Chromatogram of blank (Methanol) used in the extraction of aflatoxins injected to check its purity.



FLD: Signal A Results

Pk #	Name	Retention Time	Area	Concent
44	Aflatoxin G2	5.162	307424	2.000
46	Aflatoxin G1	6.070	524762	8.000
47	Aflatoxin B2	6.905	663997	2.000
50	Aflatoxin B1	8.287	1064133	8.000
Totals			2560316	20.000

Figure 7. Chromatogram for 20µg/L mixed aflatoxin standard solution injected to check instrument efficiency.

4.2. Analytical Quality Assurance

4.2.1. Accuracy

The accuracy of the method was determined by analysis of samples containing known aflatoxin amounts. The closeness of test results to the ―true or accepted value. The accuracy of the method was determined by spiking sample with aflatoxin standard analyzing in replicates and evaluating the recovery against the AOAC guideline for the respective concentration value (Horwitz, 2002).

4.2.2. Precision

The precision of the method was evaluated through the repeatability of the method by assaying ten replicate injections of aflatoxin mixed standard at the same concentration (20µg/kg) during the same day under the same experimental conditions to obtain an acceptable %RSD which is less than 11% as the specification required by AOAC (Horwitz, 2002).

4.2.3. Linearity

Linearity was determined by injecting a series of aflatoxin standard solutions concentration of (5, 10, 20, 30, 50, and 100µg/kg) total aflatoxin standard. The concentration and regression equation were found by plotting the peak area versus the aflatoxin concentration. And all correlation curves were with goodness of fit (R^2) >0.998, demonstrating the linearity over the concentration ranges studied.

4.2.4. Recovery

The mixture of aflatoxin standards G2, G1, B2, and B1 was spiked to 20g sample whose aflatoxin content is previously determined these spiked samples were then loaded onto the Immunoaffinity column and analyzed in duplicate by the HPLC. The method was evaluated as the percent of recovered concentration and the recovery acceptance criteria is between 70% and 120% and the %RSD was less than 11% as the specification required by AOAC. The calculated recovery was ranged from 80% to 112.0% which is within the acceptable range showing the accuracy of the method.

The %Recovery was calculated using the following formula;

$$\%\text{Recovery} = \frac{Cs - Co}{\text{Ca}} \times 100 \quad \text{.....Eq1}$$

Where;

Cs is the concentration of aflatoxin in the spiked sample in µg/kg,

Co is the concentration of aflatoxin in the original sample in µg/kg, and

Ca is the amount of aflatoxin added to the original sample in µg/kg.

4.3. Aflatoxin Determination and Calculations

After extraction and clean up, sample extracts as methanol eluate, were injected into the HPLC for the detection and quantification of aflatoxins in the sample. The aflatoxin content of the

samples was calculated using the following formula:

$$Aflatoxin\ (\mu g/kg) = n\ x(\frac{Ve}{V1})x(\frac{1}{We})xDF$$

Where;

n is amount of HPLC reading

Ve is final volume collected after elution from immunoaffinity column (µl)

VI is volume eluate injected into HPLC (µl)

We is weight of matrix represented by final extract (gm.)

DF is a dilution factor

4.4. Amount of Aflatoxins in Red Pepper Collected From Shops

Sample	**Amount of Aflatoxin in µg/kg**				
	AFG2	**AFG1**	**AFB2**	**AFB1**	**Total AF**
LS01	0.82	19.29	0.44	8.83	29.38
LS02	0.13	6.74	0.16	3.64	10.67
LS03	0.23	16.72	1.80	70.68	89.43
LS04	1.33	28.41	1.57	31.17	62.48
LS05	1.39	197.10	1.50	82.57	282.56
LS06	1.49	43.14	1.16	24.00	69.79
LS07	0.27	8.88	0.27	5.03	14.45
LS08	2.51	103.99	3.02	76.92	186.44
LS09	1.69	150.59	1.75	68.31	222.34
LS10	0.94	21.19	0.30	5.67	28.10
Mean AF	**1.08**	**59.60**	**1.20**	**37.68**	**103.92**

Table 2: Average Amounts of aflatoxin G2, G1, B2, B1 and total AF in red pepper collected from shops

The total aflatoxin content of red pepper collected from shops ranged from10.67µg/kg to 222.34µg/kg with a mean value of 103.92µg/kg. Aflatoxin B1 content ranged from 3.64µg/kg to 82.57µg/kg with a mean value of 37.68µg/kg. Aflatoxin B2 content ranged from 0.16µg/kg to 3.02µg/kg with a mean value of 1.20µg/kg. Aflatoxin G1 content ranged from 6.74µg/kg to 197.10µg/kg with a mean value of 59.60µg/kg. Aflatoxin G2 content ranged from 0.13µg/kg to 2.51µg/kg with a mean value of 1.08µg/kg. All red pepper samples collected from the shops of Addis Ababa administration have total aflatoxins exceeding the regulatory limit (10.00µg/kg) and the average contamination level is also high, 103.92µg/kg. 90% of the samples are also exceeding the limit (5.00µg/kg) by AFB1 content with high mean value, 37.68 (Table 2).

4.5. Amount of Aflatoxins in Red Pepper Collected From Exporters Site

Sample	**Amount of Aflatoxin in µg/kg**				
	AFG2	**AFG1**	**AFB2**	**AFB1**	**Total AF**
ES01	0.58	3.94	0.68	4.17	9.37
ES02	0.34	2.07	0.06	0.11	2.58
ES03	2.27	58.28	1.21	26.38	88.14
ES04	0.62	15.69	0.46	9.52	26.29
ES05	0.01	0.24	0.02	0.45	0.72
ES06	2.54	110.01	1.25	39.97	153.77
ES07	0.04	10.34	3.06	94.63	108.07
ES08	0.07	4.35	0.30	5.61	10.33
ES09	0.98	17.01	0.42	6.50	24.91
ES10	0.01	2.46	0.10	0.72	3.29
Mean AF	**0.75**	**22.44**	**0.76**	**18.81**	**42.75**

Table 3: Average Amounts of aflatoxin G2, G1, B2, B1 and total AF in red pepper collected from exporters' site

The total aflatoxin content of red pepper collected from exporters site ranged from 0.72µg/kg to 153.77µg/kg with a mean value of 42.75µg/kg. Aflatoxin B1 content ranged from 0.11µg/kg to

94.63µg/kg with a mean value of 18.81µg/kg. Aflatoxin B2 content ranged from 0.02µg/kg to 3.06µg/kg with a mean value of 0.76µg/kg. Aflatoxin G1 content ranged from 0.24µg/kg to 110.01µg/kg with a mean value of 22.44µg/kg. Aflatoxin G2 content ranged from 0.01µg/kg to 2.54µg/kg with a mean value of 0.75µg/kg. 60% of all red pepper samples collected from exporters' site of Addis Ababa administration have total aflatoxins exceeding the regulatory limit (10.00µg/kg) and the average contamination level was 42.75µg/kg. Similarly, 60% of the samples are also exceeding the limit (5.00µg/kg) by AFB1 content with mean value of 18.81 (Table 3).

The results of the analysis in table 2 &3 indicate that locally consumed red pepper samples are more contaminated by aflatoxins than red peppers prepared for export purpose. Even though the aflatoxin concentration is relatively low in red peppers ready for export than locally consumed, the average contamination amount is far from the regulatory limit.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Aflatoxin contamination is the cause of disease in humans, animals, and has aggravated loss in quality of red pepper. The amounts of aflatoxins (AFB1, AFB2, AFG1 and AFG2) were determined for both locally consumed and export red peppers using HPLC method. The HPLC system used to analyze aflatoxins gives good values of regression coefficient (R^2) values were >0.998 for aflatoxins (AFB1, AFB2, AFG1 and AFG2) calibrated by aflatoxins standard solutions in the concentration range of 5-100µg/kg. In both cases, locally consumed and export red pepper samples, most of them were contaminated with aflatoxins exceeding the European maximum permitted level (EU). These might arose due to in adequate care during harvesting, production and storage conditions and relative unfavorable humidity. Since the investigation indicates both local and export standard red peppers are highly contaminated by aflatoxins, control mechanisms should be implemented to protect consumers and exporters from harm.

5.2. Recommendations

On the basis of this research findings, I recommend that:

- It is better to conduct investigation on the factors which contribute the development of aflatoxin contamination during harvesting, production and storage of red peppers.
- Efforts has to be made to manage occurrence of aflatoxins by creating awareness and promoting good agricultural practice principles in harvesting, production and storage conditions and hazard of aflatoxin at individual and country level.
- As other countries are implementing aflatoxins control regulations mechanisms, it is highly recommended Ethiopia has to implement food standards and safety control with legally mandatory specifications of maximum limits of the toxins so as to inspect continuously.

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7. ANNEXES

Annex -1. Pictures of Sample Preparation and Analysis

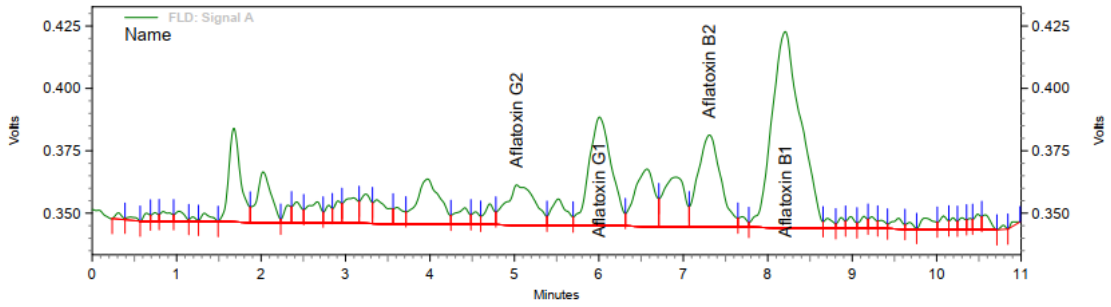
Sample Preparation

Extraction, Clean up and Analysis


KS - Oscillator

Annex-2. Chromatogram of Individual Aflatoxin in Export Red Pepper Samples

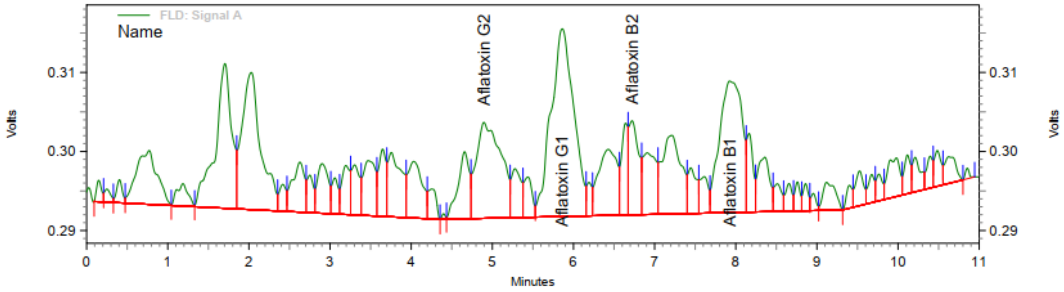
Export Red Pepper 1



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
24	Aflatoxin G2	5.026	62586	**0.411**
26	Aflatoxin G1	6.005	135541	**2.762**
29	Aflatoxin B2	7.308	114347	**0.479**
31	Aflatoxin B1	8.208	312075	**2.922**
Totals			624549	**6.575**

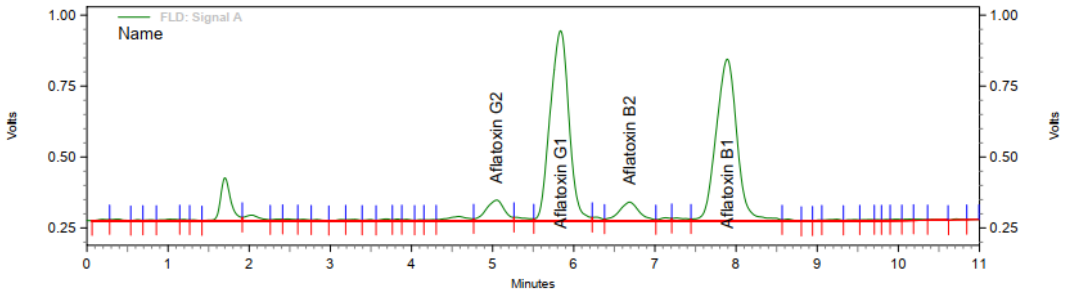
Export Red Pepper 2



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
22	Aflatoxin G2	4.896	43106	**0.237**
25	Aflatoxin G1	5.861	77764	**1.446**
29	Aflatoxin B2	6.725	16527	**0.044**
34	Aflatoxin B1	7.927	53783	**0.075**
Totals			191180	**1.802**

Export Red Pepper 3

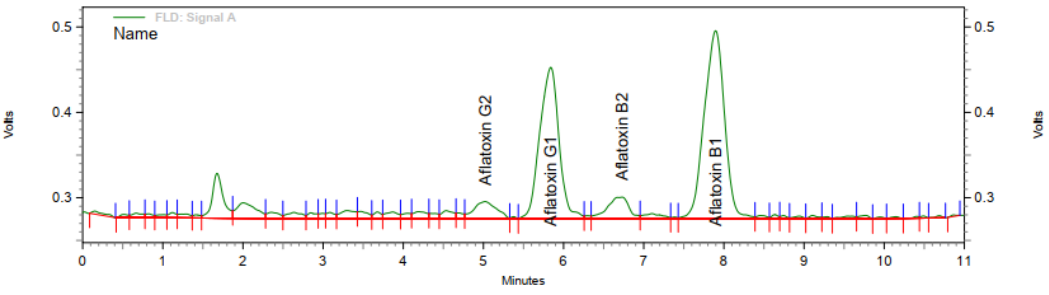


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
23	Aflatoxin G2	5.047	194477	**1.593**
25	Aflatoxin G1	5.839	1804895	**40.792**
27	Aflatoxin B2	6.689	196228	**0.844**
30	Aflatoxin B1	7.891	1722268	**18.464**

Totals		3917868	**61.692**

Export Red Pepper 4

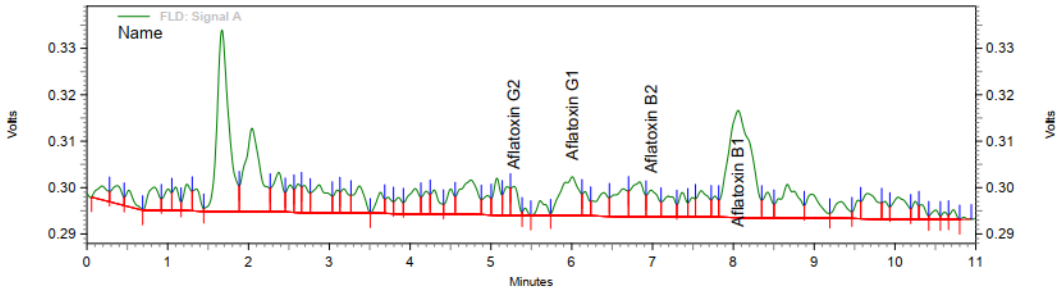


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.026	66369	**0.445**
27	Aflatoxin G1	5.839	496397	**10.983**
29	Aflatoxin B2	6.732	81261	**0.332**
32	Aflatoxin B1	7.898	652329	**6.672**

Totals		1296356	**18.432**

Export Red Pepper 5

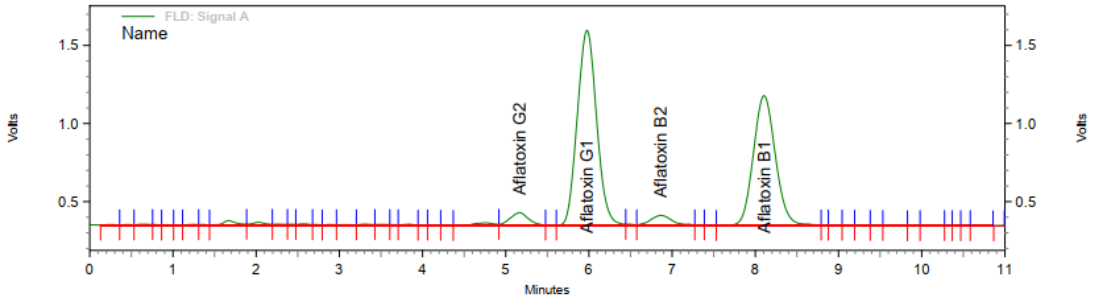


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
30	Aflatoxin G2	5.285	6268	**0.000**
33	Aflatoxin G1	6.005	21506	**0.164**
38	Aflatoxin B2	6.984	8986	**0.010**
44	Aflatoxin B1	8.057	75023	**0.310**

Totals		111783	**0.484**

Export Red Pepper 6

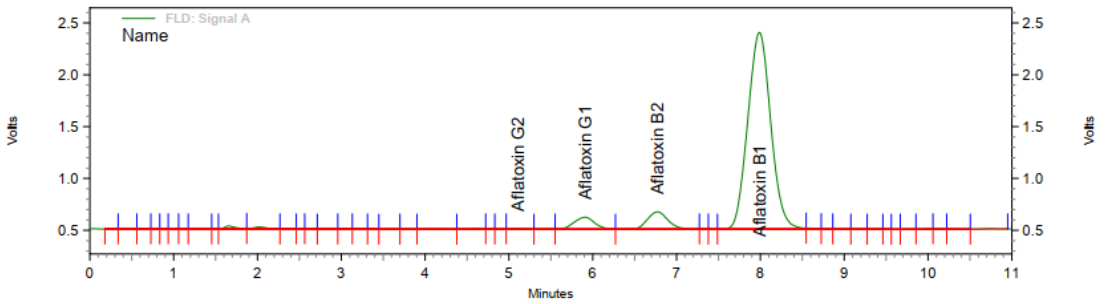


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.170	216294	**1.789**
27	Aflatoxin G1	5.976	3394926	**77.014**
29	Aflatoxin B2	6.862	204295	**0.880**
32	Aflatoxin B1	8.107	2586512	**27.988**

Totals		6402027	**107.671**

Export Red Pepper 7

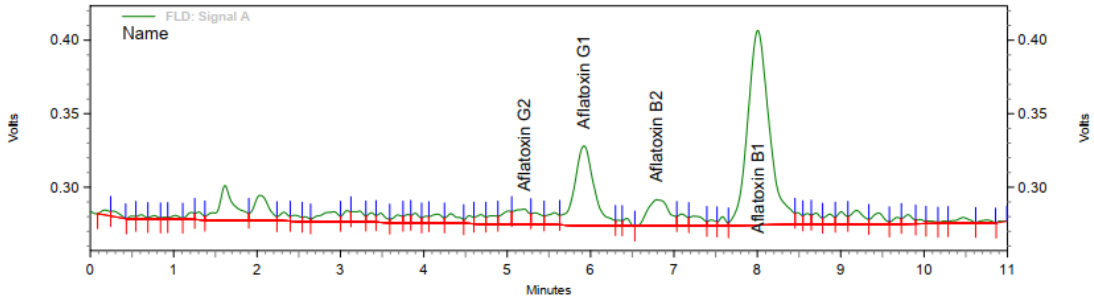


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.112	20441	**0.034**
27	Aflatoxin G1	5.911	332485	**7.249**
28	Aflatoxin B2	6.775	489119	**2.148**
31	Aflatoxin B1	7.992	6057373	**66.240**

Totals		6899418	**75.670**

Export Red Pepper 8

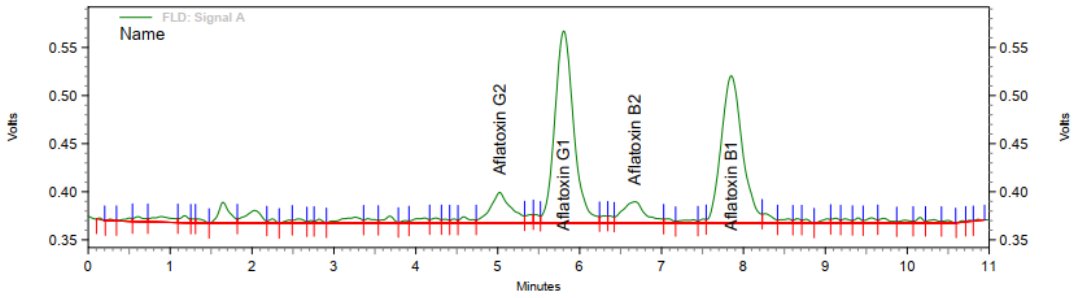


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
30	Aflatoxin G2	5.206	21765	**0.046**
33	Aflatoxin G1	5.918	147883	**3.043**
36	Aflatoxin B2	6.790	52123	**0.202**
41	Aflatoxin B1	8.006	402703	**3.921**

Totals		624474	**7.212**

Export Red Pepper 9

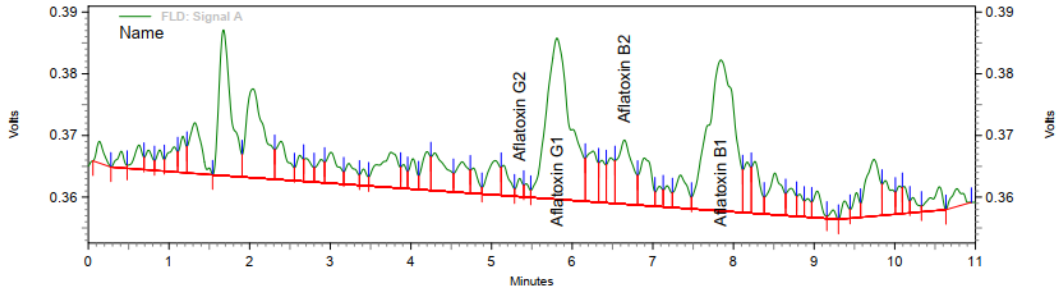


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.026	94357	**0.696**
28	Aflatoxin G1	5.803	537292	**11.914**
31	Aflatoxin B2	6.674	73070	**0.295**
35	Aflatoxin B1	7.848	460449	**4.557**

Totals		1165168	**17.463**

Export Red Pepper 10



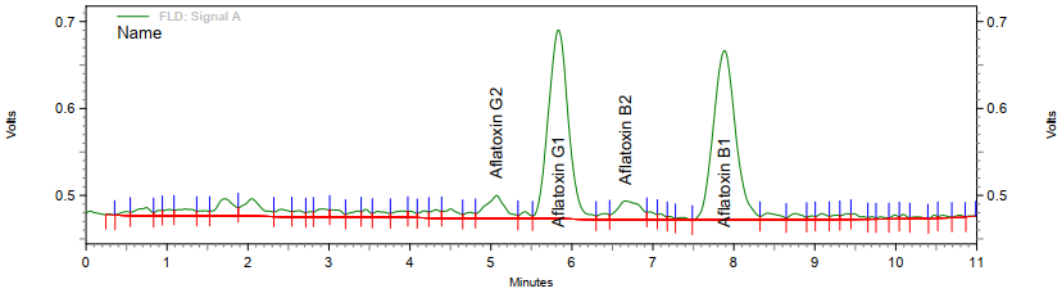
FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
27	Aflatoxin G2	5.350	2539	**0.000**
29	Aflatoxin G1	5.810	89735	**1.719**
33	Aflatoxin B2	6.646	21921	**0.068**
38	Aflatoxin B1	7.841	92491	**0.502**

Totals		206686	**2.288**

Annex-3. Chromatogram of Individual Aflatoxin in Locally Consumed Red Pepper Samples

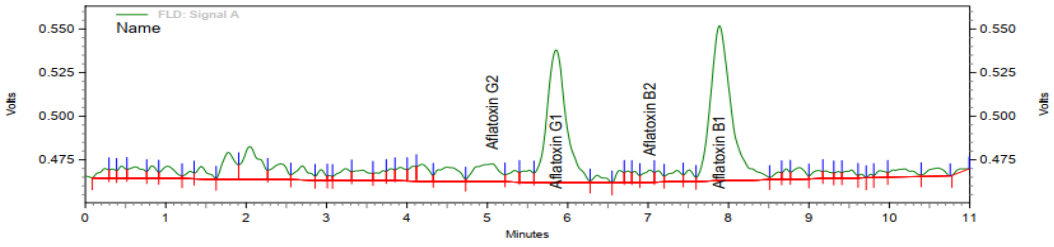
Locally Consumed Red Pepper 1



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
24	Aflatoxin G2	5.069	71407	**0.490**
26	Aflatoxin G1	5.832	622293	**13.851**
28	Aflatoxin B2	6.660	69445	**0.279**
33	Aflatoxin B1	7.884	619496	**6.310**
Totals			1382641	**20.931**

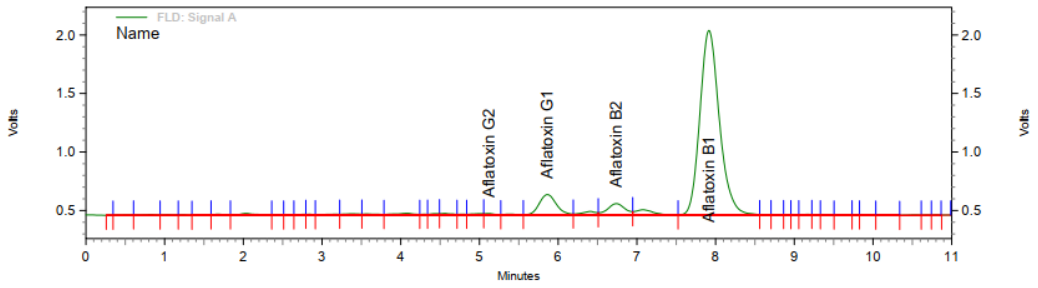
Locally Consumed Red Pepper 2



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
23	Aflatoxin G2	5.062	36517	**0.178**
26	Aflatoxin G1	5.854	210151	**4.462**
31	Aflatoxin B2	7.020	10328	**0.016**
35	Aflatoxin B1	7.884	265875	**2.413**
Totals			522871	**7.069**

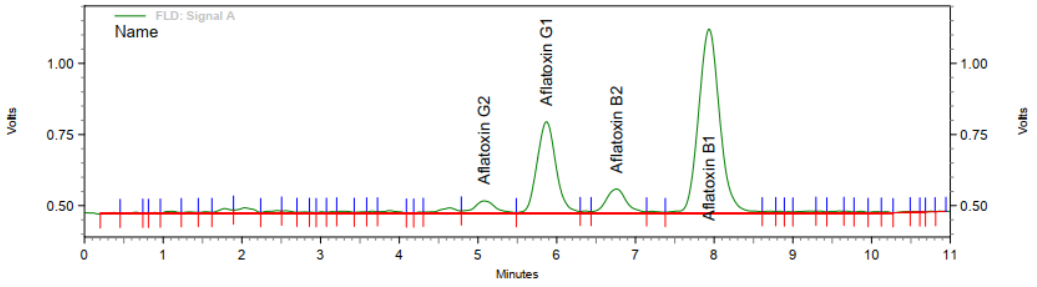
Locally Consumed Red Pepper 3



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
22	Aflatoxin G2	5.119	24361	**0.069**
24	Aflatoxin G1	5.861	464124	**10.248**
26	Aflatoxin B2	6.739	264468	**1.148**
28	Aflatoxin B1	7.913	4582443	**49.985**
Totals			5335396	**61.449**

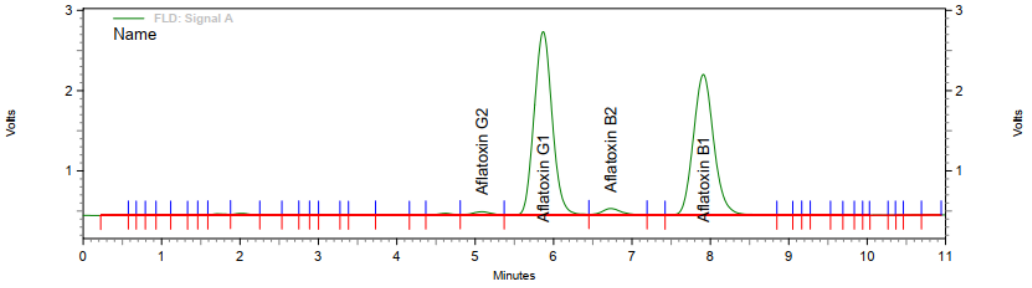
Locally Consumed Red Pepper 4



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
23	Aflatoxin G2	5.076	136899	**1.077**
24	Aflatoxin G1	5.868	897200	**20.114**
26	Aflatoxin B2	6.761	254898	**1.105**
28	Aflatoxin B1	7.934	2022981	**21.778**
Totals			3311978	**44.073**

Locally Consumed Red Pepper 5

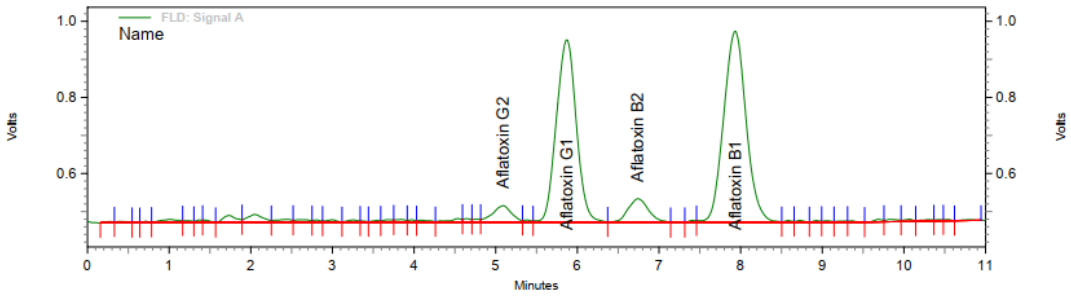


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
21	Aflatoxin G2	5.090	137902	**1.086**
22	Aflatoxin G1	5.868	6168800	**140.205**
23	Aflatoxin B2	6.732	269497	**1.170**
25	Aflatoxin B1	7.913	5358974	**58.543**

Totals		11935173	**201.005**

Locally Consumed Red Pepper 6

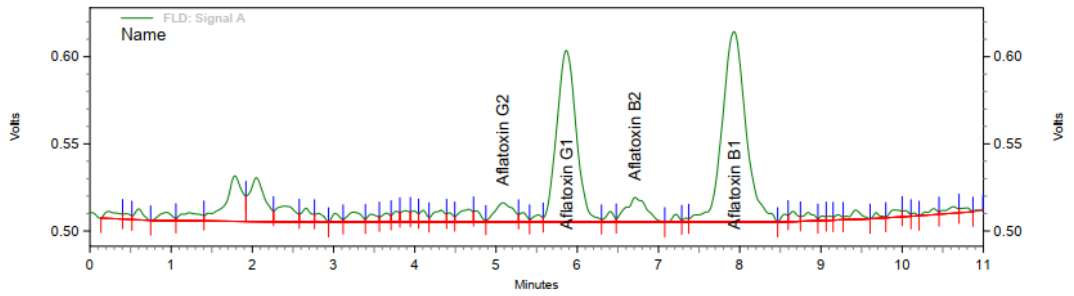


FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.090	124298	**0.964**
27	Aflatoxin G1	5.868	1375650	**31.013**
28	Aflatoxin B2	6.739	185438	**0.796**
31	Aflatoxin B1	7.934	1615998	**17.292**

Totals		3301384	**50.066**

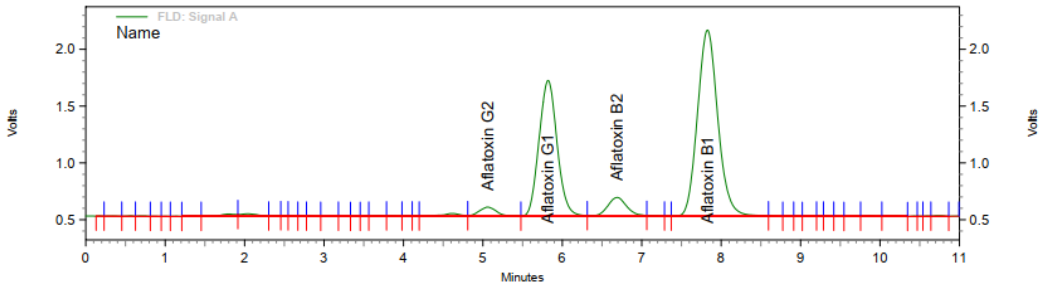
Locally Consumed Red Pepper 7



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
23	Aflatoxin G2	5.083	28823	**0.109**
26	Aflatoxin G1	5.868	275263	**5.945**
28	Aflatoxin B2	6.710	41993	**0.157**
31	Aflatoxin B1	7.934	372378	**3.587**
Totals			718457	**9.798**

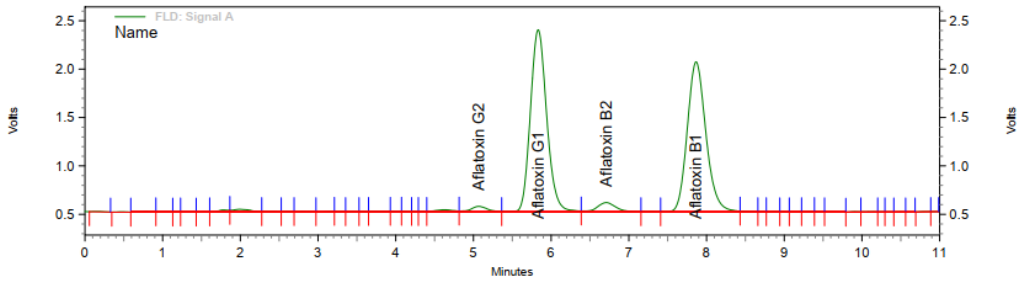
Locally Consumed Red Pepper 8



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
25	Aflatoxin G2	5.062	219733	**1.819**
26	Aflatoxin G1	5.818	3197691	**72.521**
27	Aflatoxin B2	6.689	486147	**2.135**
30	Aflatoxin B1	7.826	4933488	**53.854**
Totals			8837059	**130.329**

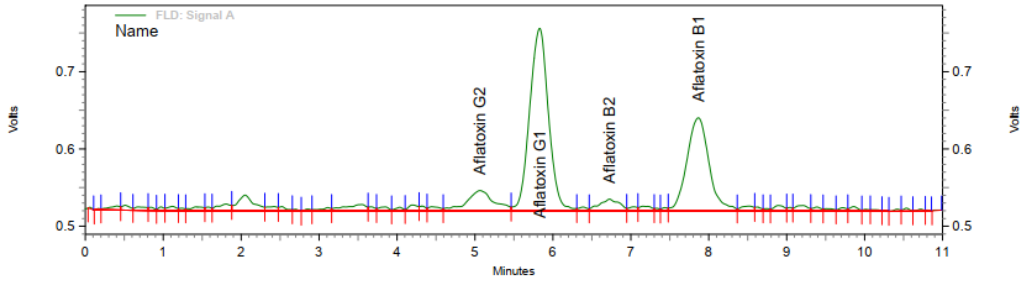
Locally Consumed Red Pepper 9



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
23	Aflatoxin G2	5.069	157049	**1.258**
24	Aflatoxin G1	5.832	4639874	**105.375**
25	Aflatoxin B2	6.710	294920	**1.283**
27	Aflatoxin B1	7.862	4372997	**47.677**
Totals			9464840	**155.593**

Locally Consumed Red Pepper 10



FLD: Signal A
Results

Pk #	*Name*	*Retention Time*	*Area*	*Concentration*
26	Aflatoxin G2	5.062	109287	**0.830**
27	Aflatoxin G1	5.832	668849	**14.911**
29	Aflatoxin B2	6.725	47300	**0.181**
34	Aflatoxin B1	7.870	402356	**3.917**
Totals			1227792	**19.839**