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Aflatoxin level in selected spices in Abu Dhabi markets

Salma Ibrahim Al Hammadi

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College of Science

Department of Biology

AFLATOXIN LEVEL IN SELECTED SPICES IN ABU DHABI
MARKETS

Salma Ibrahim Al Hammadi

This thesis is submitted in partial fulfilment of the requirements for the degree of
Master of Science in Environmental Sciences

Under the Supervision of Professor Afaf Kamal-Eldin

December 2015

Declaration of Original Work

I, Salma Ibrahim Al Hammadi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Aflatoxin Levels in Selected Spices in Abu Dhabi Markets*”, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal-Eldin, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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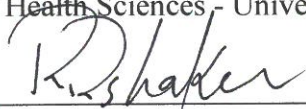
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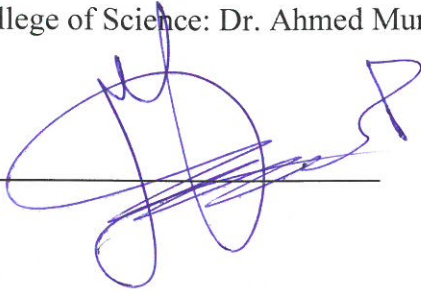
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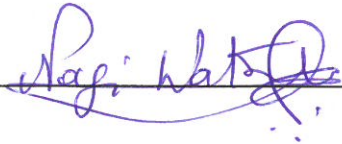


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Abstract

Aflatoxins (AF) are toxic secondary metabolites secreted by the fungi *Aspergillus* or *Penicillium*. Four aflatoxins (AFB1, AFB2, AFG1 and AFG2) are common in foods and feeds produced or stored under unfavorable conditions of humidity and temperature AFB1 is considered the most toxic.

Spices are significant ingredients in the UAE cuisine, used for the purpose of seasoning, coloring, and flavoring. In this research, 94 samples of spices (including pepper, chili, coriander, fennel seeds, paprika, turmeric, zataria, and mixed spices) purchased from different markets in the Emirate of Abu Dhabi, were analyzed for their contents of AF. Samples were analyzed using high-performance liquid chromatographic (HPLC) system, coupled with fluorescence detector. The results showed that only one paprika sample contained a level higher than the permissible limit set by the Ministry of Environment and Water (15 µg/Kg), while five other samples contained 4.3-6.7 µg/Kg of total AF. These levels are actually low compared to what was found in other countries having the same climate as the UAE. This may be due to the strict regulations implemented in the Emirate of Abu Dhabi on the process of food importation, storage and handling.

Keywords: Aflatoxins, *Aspergillus* sp., spices, Abu Dhabi, UAE

Title and Abstract (in Arabic)

مستوى الأفلاتوكسينات في التوابل من منتقاة من أسواق أمارة أبوظبي

ملخص

الأفلاتوكسينات (AF) هي مركبات ثانوية سامة يفرزها الفطر اسبرجيلوس أو الفطر البنسليونيوم (*Aspergillus or Penicillium*) و من أكثر أنواعا لأفلاتوكسينات شيوعاً في الأغذية و الأعلاف هي AFB1, AFB2, AFG1 , AFG2 و التي تتكون في الأغذية المنتجة أو المخزنة تحت الظروف الدافئة الرطبة. كما يعتبر AFB1 الأكثر سمية.

التوابل هي من المكونات الهامة في المطبخ الإماراتي ، حيث تستخدم لغرض التلوين، أو إضافة النكهة في الأطعمة، في هذا البحث، تم تحليل 94 عينة من التوابل بما في ذلك الفلفل الأبيض و الأسود والفلفل الأحمر، والكزبرة، بذور الشمر، والفلفل الحلو، والكرم، الزعتر، والتوابل المخلوطة. وقد تم جمع هذه التوابل من أسواق مختلفة في إمارة أبوظبي و ذلك للتأكد من احتوائها أو عدم احتوائها على افلاتوكسينات (AF). وقد تم عمل دراسة باستخدام نظام كروماتوغرافيا سائلة عالية الأداء HPLC.

وكانت نتائج البحث كالتالي عينة واحدة فقط من الفلفل الحلو تحتوي على 17 مايكروجرام /كيلو غرام من AF، و هي أعلى من الحد المسموح به وفقاً لقرار وزارة البيئة والمياه بشأن مستوى أفلاتوكسين في المواد الغذائية 10 مايكروجرام /كيلو غرام بينما احتوت خمسة عينات على مستوى أفلاتوكسينات ما بين 4.3 - 6.7 مايكروجرام /كيلو غرام، و تعتبر هذه المستويات منخفضة إذا ما قورنت بالمستويات التي تم التوصل إليها بالدول الأخرى التي لها نفس مناخ دولة الإمارات العربية المتحدة. وهنا يظهر مدى صرامة اللوائح المنفذة في إمارة أبوظبي فيما يختص بعمليات استيراد المواد الغذائية وتخزينها والتعامل معها. لضمان سلامة الغذاء والحفاظ على المستهلك.

مفاهيم البحث الرئيسية: افلاتوكسين، اسبرجيلوس، توابل، أبوظبي، الإمارات العربية المتحدة

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Last but not least, I would like to thank my beloved parents for being a beacon of guidance throughout my life and for nurturing me to love scientific adventures. Special heartily words to my sweet daughter Mariam for refueling my motivation with her innocent smiles and bright eyes.

Dedication

To my beloved parents and sweet daughter Mariam

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Chapter 1: Introduction

2.1 1.1 Overview

The United Arab Emirates (UAE) strives to be one of the leading countries in many aspects. This includes the assurance that the foods imported, marketed, and consumed in the country are safe according to the Food Law No.2, (2008). The UAE imports high amounts of all kinds of foods including spices, which are often stored for variable lengths of time before consumption. Therefore, checking food quality and implementing the necessary food control standards are necessary to make sure that the quality and safety of food are not compromised during production, transportation, and/or storage. Moreover, the weather in UAE is typically a desert climate; being hot and humid about eight months a year. Such climate conditions are favorable to cause microbial contamination of some stored food materials (Regulation No (6) of 2010 Food hygiene throughout the Food Chain, 2010, UAE claimant, 2015).

In UAE, most of the cases of microbial food spoilage and contamination are being caused by mycotoxins. Since such microbial contamination might cause severe loss of the national strategic food storage or present a food safety concern, this has drawn attention to conduct investigations especially on mycotoxins as a serious food contaminant. As a result, legislations and regulating guidelines were introduced and they have played vital roles in improving the conditions of food storage and consumption in a way to assure safety, in order to warrant consumer trust (Food Law No.2, 2008, Regulation No (6) of 2010 Food hygiene throughout the Food Chain, 2010).

Spices are important ingredients in the UAE cuisine, where they are commonly used as seasoning for coloring, adding flavor, and delectable aromas to the food (Hashem & Alamri 2010). This study aims to screen the presence of the four aflatoxins of AFB1, AFB2, AFG1, and AFG2 in major spices sold in the Emirate of Abu Dhabi, UAE. The study places special emphasis on eight common types of spices, namely pepper, chili, coriander, fennel seeds, paprika, turmeric, zataria, and mixed spices as shown in (Table 1) (Hashem & Alamri 2010).

Table 1: Spices studied in this thesis with their English and Latin names

No .	English name	Latin name	Family	Used part	Code
1	Chili	<i>Capsicum annuum L</i>	Solanaceae	Fruit	C
2	Coriander	<i>Coriandrum sativum</i>	Umbelifers	Seeds	Co
3	Fennel seeds	<i>Foeniculum vulgare</i>	Umbelifers	Seeds	F
4	Paprika	<i>Capsicum annuum L</i>	Solanaceae	Dried fruits	P
5	Pepper	<i>Piper nigrum</i>	Piperaceae	Black pepper: Dried fruits	BP
				White pepper: Dried fruits after removing black husk	WP
6	Turmeric	<i>Curcuma longa L.</i>	Zingiberaceae	rhizomes	T
7	Zataria	<i>Zataria multiflora Boiss</i>	Lamiaceae	Leaves	Z
8	Mixed spices	mixed of common type of spices like pepper, coriander, fennel			MS

1.2 Relevant Literature

Mycotoxins are toxic secondary metabolites produced by the fungi *Aspergillus spp.*, *Penicillium spp.* and *Fusarium* (Table 2). The species of fungi-

producing toxins during harvest is *Fusarium*, which is classified as field fungi (Riordan & Wilkinson 2008, Santos *et al.* 2011) while *Aspergillus spp.* and *Penicillium spp.* known as mould or “storage mould”, produce mycotoxins during and/or after harvest. *Penicillium* and *Aspergillus* species are the most common fungi and aflatoxins (AF) are the most common among the toxic substances secreted by these fungi (Santos *et al.* 2011, Tančinová *et al.* 2014). The designation of aflatoxins as acronym for these substances came from the name of mushrooms toxin, where **A** is taken from the word (*Aspergillus*) and **fla** is taken from the word (*flavus*) adding *toxin* in the end (Rawal *et al.* 2010, Atas *et al.* 2012).

The conditions conducive to the growth of *Aspergillus* and secretion of AF include heat, high humidity, and poor ventilation conditions (Hashem & Alamri 2010, Muller & Basedow 2007, Ozbey & Kabak 2012). Therefore, these moulds are mainly found in tropical and subtropical areas with moisture higher than 12% and temperatures around 21° C (Hammami *et al.* 2014). Also, the type of food material plays a key role in the growth of these fungi, where high content of fatty substances, proteins, and carbohydrates increase the opportunity to produce mycotoxins. Thus, the higher the exposure to heat and humidity during transport and storage of agricultural crops, the more possibility that these crops are contaminated with mycotoxins (Muller & Basedow 2007, Ozbey & Kabak 2012).

Table 2: Names of fungi and their produced mycotoxins

Fungus	Mycotoxin
<i>Fusarium</i> (Field fungi)	
<i>F. graminearum</i>	<i>Trichothecenes</i>
<i>F. culmorum</i>	<i>HT-2; type B: DON, NIV</i>
<i>F. crookwellense</i>	<i>Zearalenone</i>
<i>Aspergillus</i> (storage mould)	
<i>A. Flavus</i>	<i>Aflatoxin B₁, B₂, G₁, G₂</i>
<i>A. Parasiticus</i>	<i>Cyclopiazonic acid</i>
<i>A. Versicolor</i>	<i>Sterigmatocystin</i>
<i>A. Ochraceus</i>	<i>Ochratoxin A</i>
<i>Penicillium</i> (storage mould)	
<i>P. verrucosum</i>	<i>Ochratoxin A, Citrinin</i>
<i>P. aurantiogriseum</i>	<i>Pencillic acid, Citreoviridin</i>
<i>P. citrinum</i>	<i>Cyclopiazonic acid, Penitrem A</i>
<i>P. expansum</i>	<i>Patulin</i>

The foods most susceptible to aflatoxins contamination include peanuts, different kinds of grains (e.g. wheat, barley, corn, rice) seeds (e.g. soybeans, cottonseeds, sesame, pumpkin seeds and sunflower), nuts (e.g. pistachios, walnuts, almonds, hazelnuts, cashews), as well as coffee, milk, baby foods, dried fruits, fresh vegetables, and other dried foods (Riordan & Wilkinson 2008, Hashem & Alamri 2010, Ozbey & Kabak 2012). In addition, AF may be found in spices and herbs, which are very important ingredients used in different types of food and/or medicine

all around the world (Hashem & Alamri 2010, Mahgubi *et al.* 2013, Hammami *et al.* 2014).

1.3 Common types of Aflatoxins

More than 300 species of mycotoxins were identified in foods including 20 types of AF. The most prominent types are aflatoxins AFB1, AFB2, AFG1, and AFG2 (Figure 1). The distinction between B and G aflatoxins came as a result of their color upon exposure to UV light, which turns B aflatoxins **Blue** and G aflatoxins **Green** (Rawal *et al.* 2010, Santos *et al.* 2011, Kalkan *et al.* 2011, Ozbey & Kabak 2012, Mushtaq *et al.* 2012,).

The consumption of contaminated food and feeds is the main source of exposure to AF for both humans and animals, e.g. through vegetable products and cereal grains (Bianco *et al.* 2012). When AFB1 and AFB2 are consumed, they undergo metabolism by liver enzymes to mainly produce the metabolites aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2), respectively. AF can also be ingested through plant foods, milk, meat, and eggs; for example, aflatoxin B1, M1, B2, and Aflatoxicol were found in eggs of Japanese Quails given 25, 50 and 100 micrograms of contaminated feed for 90 days (Peterson *et al.* 2006).

The United Nation Food and Agricultural Organization (FAO) estimated that 25% of the world's foods are contaminated with moulds that produce mycotoxins (Muscarella *et al.* 2007). However, contamination of milk by AFM1 is considered to pose a carcinogenic risk but less serious than contamination with AFB1. AFM1 could be isolated from cow's milk 12 hours after eating contaminated feed and is not affected by pasteurization processes and remains in dairy products like cheese, yoghurt, cream, etc. (Muscarella *et al.* 2007, Brankov, Jovanovic, & Grujic, 2013).

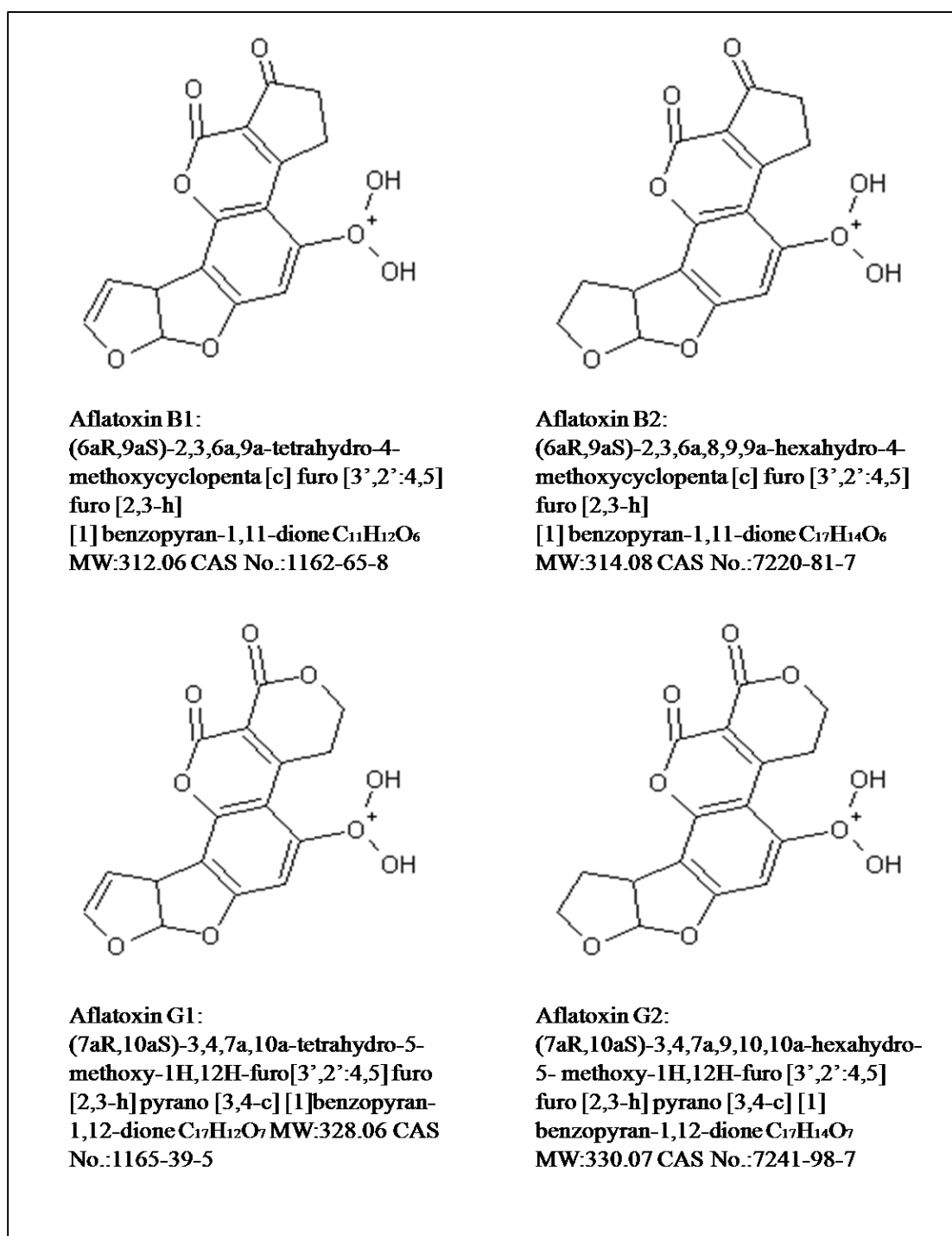


Figure 1: Chemical structures of aflatoxins AFB1, AFB2, AFG1 and AFG2

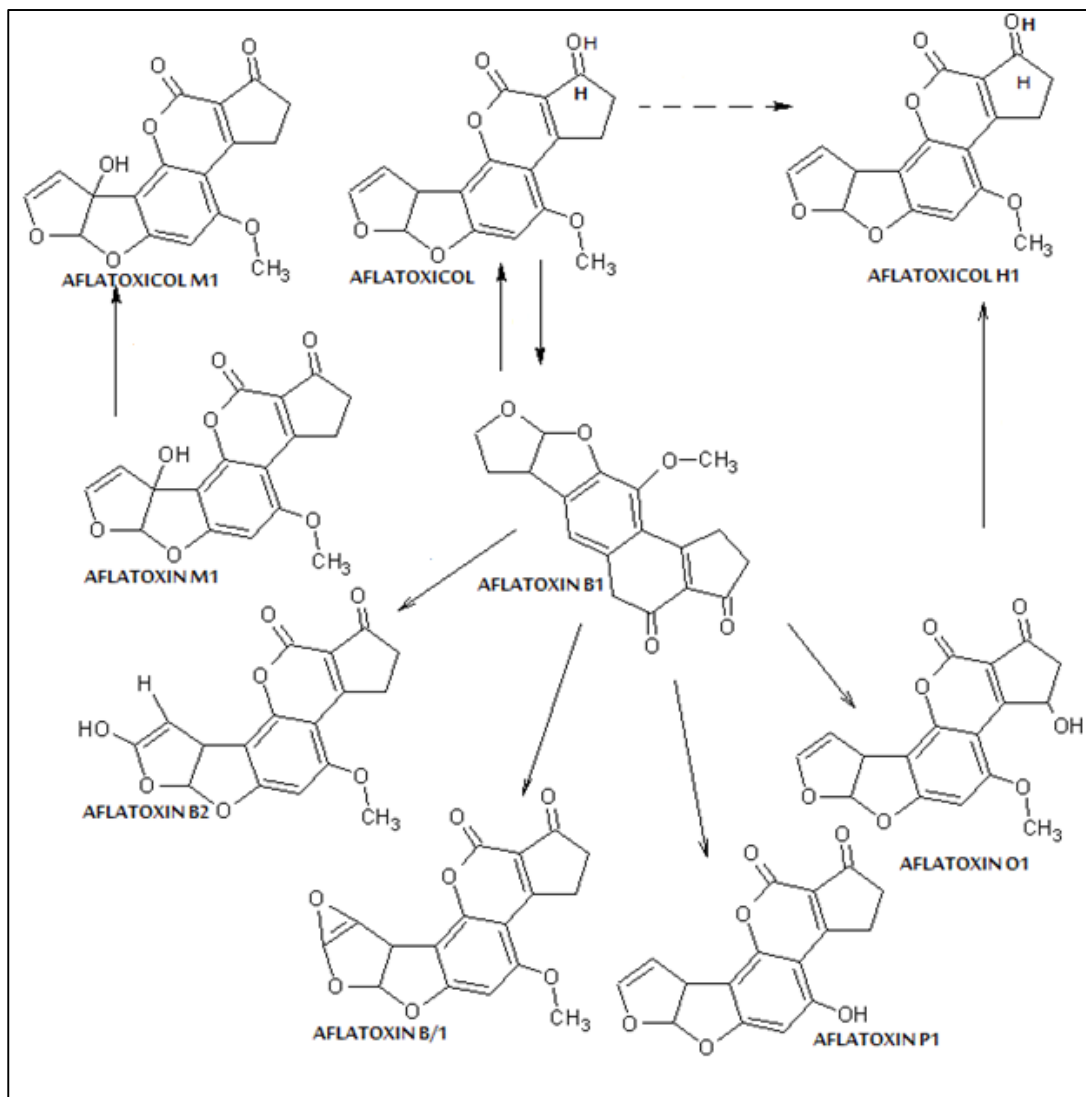


Figure 2: The metabolism of aflatoxins by liver enzymes

1.4 Toxicology

The symptoms of poisoning with aflatoxin "*aflatoxicosis*" include necrosis of the liver, bile duct proliferation, oedema, and lethargy. The amount of aflatoxin in human exposure leading to toxicity is different between adults and children.

Children are particularly more susceptible to poisoning and at lower AF levels (Mahgubi *et al.* 2013, Williams *et al.* 2014). Aflatoxin toxicity is most dangerous to the liver, since it can affect the metabolism of proteins and fats, which could cause liver cancer as a result of fat deposition in the liver and damaged liver

cells. A connection between the number of incidence of hepatitis (B) and consumption of foods contaminated with AF has also been shown in studies conducted e.g. in China and Africa (Sindhu *et al.* 2011, García-Cela *et al.* 2012).

Other studies found an association between virus hepatitis B and the incidence of liver cancer. Studies conducted in South Africa found that children infected with the hepatitis C virus because of consumption of foods contaminated with AF, like peanut butter, could get liver cancer 20 - 30 years later. Therefore, the health services in South Africa recommended that ingested foods should contain no more than 10 micrograms of aflatoxins/ kg (Williams *et al.* 2014). Studies have also indicated that exposure to aflatoxin by breathing into the lungs leads to lung cancer (Williams *et al.* 2014).

Therefore, it is essential for countries importing foods to pay special attention so that the level of AF contamination in these foods should not exceed the limits recommended by local authorities or international organizations such as the *Codex alimentarius*. In addition, public health authorities in a given country must not forget the transport and storage conditions of such foods after importing them. If appropriate conditions are not observed, the levels of aflatoxin in food may increase further (Molyneux *et al.* 2010).

The harmful effects on human and animal health plus its importance in international trade are the reasons behind the global significance of aflatoxin contamination of food. Aflatoxin's identification as a Class 1 carcinogen by the International Cancer Research Institute helped to lower AF concentrations significantly especially in traded goods. The tolerance limit assigned by the Indian

Government under the Prevention of Food Adulteration act is 30 μ g/Kg (Asim *et al.*2011).

1.5 Maximum Permitted level in foods

Many countries have now set maximum permitted aflatoxin levels to ensure that farmers and processors follow practices that control (Table 3) hygiene and storage conditions to prevent contamination (Punam & Dhiraj, 2015).

Table 3: Maximum permitted levels of total aflatoxins in spices

#	Country	Permissible levels	Reference
1	UAE	10 μ g/Kg for total aflatoxins	(Ministry of Environment and Water Decision)
2	South Africa	5 μ g/Kg for B1 and from 10 μ g/Kg for total aflatoxins.	(Williams <i>et al.</i> 2014).
3	European countries	5 μ g/Kg for B1 and from 10 μ g/Kg for total aflatoxins.	(Atas <i>et al.</i> 2012)
4	USA	20 μ g/Kg for total aflatoxins	(Kalkan <i>et al.</i> 2011)
5	India	30 μ g/Kg for total aflatoxins	(Asim <i>et al.</i> 2011)

1.6 Factors affecting fungal growth and production of aflatoxins

A. The fungi strain

Aflatoxins are produced by many fungi strains such as *Asparagillus flavus*, *A. parasiticus*, *A. oryza*, *A. Falvus* in addition to other species that can produce more than one type of aflatoxins such as *A. rubber*, *A. niger*, *A. wentii*, *Penicillium puberulum* and others, which produce more than 1,500 secondary representative

materials. About 30% of the aflatoxins are produced by strains of *A. flavus* while *P. Puberulum* is one of the most persistent fungi in agricultural crops and food grains (e.g., rice, corn, barley, peanuts, and walnuts). Some food products such as bread and products of similar structure, and some fermented products are stored under appropriate conditions of humidity and temperature favoring the growth of AF production fungi (Peterson *et al.* 2006). The fungus *A.falvus* resides in damaged tissues of agricultural crops and may stay inactive during storage until the conditions are appropriate for it to grow and produce AF (Riordan & Wilkinson 2008, Ozbey & Kabak 2012).

B. The substrate and its nature

In general, grains belonging to the grass family, such as wheat and rice, are considered suitable food for the production of toxins by fungi in contrast to less spread of fungus in dry oil seeds such as cottonseeds, soya beans and peanuts. This may also be because of their high content and the ease of utilization of carbohydrates by fungi. A comparative study conducted using three strains of *A. falvus* producing AF in different plants found that the fungi growth and mycotoxins production were greater in corn, wheat and rice than in peanuts and soybeans (Muller & Basedow 2007).

C. Relative humidity and temperature

The critical moisture content in foods conducive to aflatoxin production is equivalent to a relative humidity of 65 to 70 percent. Below this humidity, only a mini scale amount of fungi can grow and mycotoxins poisoning poses no threat (García-Cela *et al.* 2012). However, higher moisture limits are dangerous when storing different types of plant foods (Mahgubi *et al.* 2013).

D. Aeration

Fungi need oxygen for their vegetative growth and germs configurations, and they vary in their ability to withstand high concentrations of carbon dioxide. It was shown that most fungi cannot grow at oxygen concentrations below 1-2% (Abdel-Aziema *et al.* 2011). Generally, reducing the oxygen concentration to 1% with an increase in carbon dioxide to 80% will inhibit the production of AF by the fungus *A. falvus* (Peterson *et al.* 2006, Abdel-Aziema *et al.* 2011).

E. Damage

It was observed that the damage of fruits and seeds while they are in the agricultural land or during the process of harvesting and mechanical processing is linked to infection with *A. falvus* and AF production. Mechanic damage can easily cause fungal infection and production of mycotoxins inside the fruit because the outer shell of the fruit acts as a contraceptive against fungal infection. Moreover, it was found that insects play an important role in the process of grain damage and as carriers and tankers for the fungus. In addition, the fungus *A. falvus* is carried by more than 10 kinds of insects known to infect food grains (Muller & Basedow 2007, Atas *et al.* 2012).

F. Handling

Factors such as poor sanitation, handling, transportation, processing, and improper storage enhance the incidence of aflatoxin contamination (Jeswal & Kumar, 2015). The contamination of spices can occur due to pre-harvest, postharvest, and storage conditions (Arslan *et al.* 2013). Consequently, caution is necessary during such processes to hinder the production of harmful mycotoxins (Jeswal & Kumar, 2015). Generally speaking, food obtained from open markets have

higher levels of contamination than those collected from supermarkets. Evidently, the risk of AF contamination is higher in non-sanitary conditions. It is possible that spices get contaminated by AF in open-air markets due to dust, improper handling, and storage as well as unhygienic environments. The fact that most products sold in open bazaars remain unpacked also exposes them to microbial contamination (Arslan *et al.* 2013).

1.7 Spices and levels of Aflatoxin contamination

(Table 4) presents data on total aflatoxins levels in selected spices, which will be investigated in this thesis.

A. Pepper (*Piper nigrum*)

Pepper is the most common spice used globally. Different studies on the mycotoxin levels in this spice reveal a consensus that black pepper has one of the highest toxin concentrations compared to other spices (Jeswal & Kumar, 2015, Vasanthi & Ramesh, 2014). For instance, Siruguri and Bhat (2014) referred to 16 different studies conducted on black pepper whose results showed that the spice has AF levels in the range <1-60 µg/Kg (Vasanthi & Ramesh, 2014). A study of five spices purchased from local markets in Qatar including black pepper revealed that some samples of spice exceeded the maximum allowed AF levels (Hammami *et al.* 2014).

Table 4: Examples of levels of aflatoxin in different types of spices

Type of spices	Country	level of AF($\mu\text{g/mL}$)	Reference
Chili	Irish	3	(Riordan & Wilkinson, 2008)
	Turkey (Red chili flake)	2-14.3	(Ozbey & Kabak, 2012)
	Turkey (Red chili powder)	0.2 -37.4	(Ozbey & Kabak, 2012)
	Pakistan (Red pepper)	3.3	(Arshad <i>et al.</i> 2012)
Coriander	Pakistan	3.3-6.6	(Arshad <i>et al.</i> 2012)
	Irish	0.3	(Riordan & Wilkinson, 2008)
Paprika	Irish	0.3	(Riordan & Wilkinson, 2008)
Pepper	Irish	0.4	(Riordan & Wilkinson, 2008)
	Turkey	0.1 - 0.4	(Ozbey & Kabak, 2012)
	Qatar	N.D.	(Hammami <i>et al.</i> 2014)
	Pakistan	3.7	(Arshad <i>et al.</i> 2012)
Turmeric	Irish	2	(Riordan & Wilkinson, 2008)
	Pakistan	6.5	(Arshad <i>et al.</i> 2012)
	Qatar	13.2	(Hammami <i>et al.</i> 2014)

ND: not detected

White pepper and black pepper varieties are used in similar ways in cooking but food flavored with white pepper is generally much hotter. As mentioned earlier, the environmental and processing conditions of a spice increase or lessen the incidence of aflatoxin contamination, and pepper is no exception. Scholars are yet to conduct comprehensive studies of the aflatoxin risk in white pepper. However, the

existing literature shows that the level of contamination in white pepper is not as high as that found in black pepper and chili. For instance, it was observed that the aflatoxin level found in crushed white pepper was less than the maximum limit, which made it much safer for human use (Tančinová *et al.* 2014).

B. Chili (*Capsicum annum L.*)

Several studies demonstrated that chili has a high probability of aflatoxin contamination. According to a review of 28 different studies conducted between 1975 and 2013, the aflatoxin level in chili ranged from <1-969 µg/Kg (Vasanthi & Ramesh 2014). A study conducted on chili powder samples also revealed that there was a high level of aflatoxin in the spice (Hammami *et al.* 2014). People can use raw spices in their food or they can sometimes cook them without processing, which poses a great health hazard. In a separate study conducted on 80 chili samples sold in Malaysian supermarkets and open markets, it was found that 65 percent of the samples had high levels of aflatoxin contamination (Jalili & Jinap 2012). The highest incidence in the chili was with AFB1 (Arslan *et al.* 2013).

Studies have shown that paprika samples collected from Hungary, Peru, Portugal, and Turkey were highly contaminated with aflatoxins (Hernández-Hierro *et al.* 2009, Kursun & Mutlu, 2010, Gülderinet *al.*2012). In 2004, the European Commission discovered that Hungarian paprika had aflatoxin levels that surpassed the EU limits and ordered its withdrawal from the European market (Hernández-Hierro *et al.* 2009). A study on paprika marketed in Spain, on sample grown and produced in Brazil, Peru, Spain and Zimbabwe showed that the samples from Peru had the highest AF contamination, whereas the ones from Brazil and Zimbabwe had very low levels (Hernández-Hierro *et al.* 2009). Another study on 21 samples of

paprika powder from retail outlets in Spain showed that 90 percent of the samples had AFB1 contamination though at lower levels than the limit of 5 µg/Kg (Hernández-Hierro *et al.*2009). Obviously, the incidence of aflatoxin contamination in spices is not specific for certain countries, but the fungi thrive in particular climatic conditions. As mentioned before tropical climate areas generally have favorable conditions of humidity and temperature for microbial growth as well as the production of mycotoxins (Arslan *et al.* 2013).

C. Coriander (*Coriandrum sativum*)

Coriander seems to have a lower AF risk than black pepper. Studies on coriander seeds showed that their AF levels ranged between <1-16µg/Kg (Vasanthi & Ramesh 2014). Some samples of coriander had AF contamination levels that were above the maximum limit, as with other spices, the findings from experiments conducted on diverse coriander samples indicated that AF contamination depends on the growing, harvesting conditions as well as storage (Arshad *et al.* 2012). In particular, delays in drying of agricultural products or excessive moisture and a tropical climate can result in higher AF contamination. Because of this dependency, it is difficult to extrapolate findings in particular studies for establishing universal levels for each spice or place these spices in a hierarchy of AF risk making it difficult to infer the AF level in specific species from these studies (Arshad *et al.*2012).

D. Fennel Seeds (*Foeniculum vulgare*)

Fennel seeds are also susceptible to aflatoxin contamination. According to a study conducted on fennel samples obtained from India, 45.4 percent of the samples

contained *Aspergillus flavus*. However, these researchers found that fennel has a relatively low AF risk and less contamination than black pepper (Jeswal & Kumar, 2015, Vasanthi & Ramesh 2014). Again, the level of contamination still depends highly on how the crop is grown, harvested, handled, stored, and processed. Unhygienic conditions and a humid climate result in the proliferation of *Aspergillus* fungi that can attach to fennel seeds that produce aflatoxin, which remains stable under heat or normal processing of fennel seeds (Vasanthi & Ramesh 2014).

E. Turmeric (*Curcuma longa*)

Turmeric has a medium aflatoxin risk. It showed lower levels of microbial contamination than black pepper and chili and AF levels ranging between <1-9 µg/Kg (Vasanthi & Ramesh 2014). On the other hand, a study conducted on turmeric samples collected from Doha revealed that they exceeded B1 and the total aflatoxin maximum permitted levels (Hammami *et al.* 2014). It was also found that the presence of toxins in turmeric samples obtained from India was alarming (Jeswal & Kumar, 2015).

F. Zataria (*Zataria multiflora*)

Zataria is a traditional spice used in food and in flavoring yoghurt. Few studies have investigated the levels of aflatoxin contamination in zataria and have showed that zataria has an inhibitory effect on the growth of these fungi (Shokri *et al.* 2011). Gandomi *et al.* (2010) tested the effect of zataria essential oil on *Aspergillus flavus* and demonstrated that the essential oil-suppressed the size of fungal colonies and affected the processes involved in aflatoxin production. The explicit position derived from these findings is that zataria spice has components that can suppress fungi. Since the spice can limit fungal colony expansion and mutation, it is also possible that it has

reduced levels of accumulated aflatoxin. Today, there is an increasing body of scholarly research on zataria essential oil as a potential means of preventing aflatoxin production.

Chapter 2: Methods

2.2 Material Sampling

Ninety-four samples were collected from nine various supermarkets in Abu Dhabi City including 17chili,10 coriander, 8 fennel seeds, 6paprika, pepper (9 black pepper, 8white pepper), 9turmeric,16 zataria, and 11mix spices.

Table 5: Details of samples collected from nine supermarkets in Abu Dhabi

Country	M 1	M 2	M 3	M4	M 5	M 6	M 7	M 8	M 9	Total
India	1 C# 1 P* 1 T# 1 WP* 1 MS#	-	2 C# 1 Co* 1 Co# 1 F* 1 F#	2 C# 2 T# 1 MS#	1 BP* 1 C* 1 T*	1 C# 1 F* 1 T#	1 BP* 1 Co# 1 F#	2 Co# 2 Co* 1 F# 1 F*	-	31
Jordan	-	-	-	-	-	1 BP# 1 P# 3 Z# 1 Z*	-	-	-	6
Lebanon	-	-	1 Z*	-	-	1 Co# 1 P# 2 Z#	-	1 F# 1 Z*	1 Z*	8
Pakistan	-	-	1 BP# 1 C#	1 MS#	-	-	-	-	-	3
Syria	-	-	-	-	1 P# 1 WP* 1 MS*	1 F# 1 Z*	-	-	2 Z#	7
Other		1 C# 1 T# 1 WP# 1 MS#	1 BP* 1 C# 4 C* 2 P# 2 T# 1 WP# 1 WP* 1 MS# 2 MS*	3BP# 3 C# 2WP# 1 MS#	1 T* 1 M*	1 Co# 1 Co* 2 Z* 1 BP# 1 WP# 1 MS#		2Z#		39
Total	5	4	24	15	8	22	3	10	3	94

Markets (M), Black pepper (BP), Chili (C), Coriander (Co), Fennel(F), Paprika (P), Turmeric (T), White pepper (WP), Zataria (Z), Mixed spices (MS). Non-packaged (*), packaged (#)

2.3 Sample Preparation

Grinded, homogenized samples of spices (25g) were mixed with sodium chloride (5g) and extracted with 100 ml of aqueous methanol (Methanol/Water; 80/20v/v) to prepare the samples for the recent study. After shaking for 1 hour, centrifugation, and filtration, 10 ml aliquot of the aqueous methanol extract was

diluted with 40 ml deionized water and 4 ml of the diluted sample (equivalent to 0.2g sample) was subjected to further clean up using solid phase extraction.

The 4ml of the extract were mixed with 12 ml of phosphate buffered saline (weight: 1814.5 - 2005.5 mg/tab, pH: 7.20 - 7.60) (Sigma-Aldrich Saint Louis, USA). Thereafter, the mixture was applied to the solid phase extraction column (1g) (AflaTest Column / VICAM USA) and the column was washed using 20 ml distilled water at a rate of 1.5 ml/min, and thereafter, the AF were immobilized and eluted with 1 ml HPLC-grade methanol (J.T.Baker[®] Deventer, Netherlands). The eluted aflatoxins were filtered through a 0.45 µsyringe filter for analysis by high-performance liquid chromatographic (HPLC).

2.4 HPLC Analysis and Recovery

The extracts were then analyzed by a HPLC system (Model 2695, USA), coupled with fluorescence detector (Model 2475, USA). The Mobile phase was water: methanol: acetonitrile (5:1:1, v/v/v) containing 70 µl nitric acid and 0.21g potassium bromide. The flow rate was 0.5mL/min, the column temperature was 30°C, and the injection volume was 40µL. The separation of AFB1, AFB2, AFG1, and AFG2 was performed on a C18 column (Agilent Zorbax Bonus RP 3.5µm 2.1x 150mm). After separation, aflatoxins AFB1 and AFG1 were photo-chemically derivatised by irradiation with UV light at 254 nm, to allow their detection with a fluorescence detector (excitation: 360nm, emission: 440nm). The photochemical derivatisation has no influence on the measurements related to aflatoxins AFB2 and AFG2. Separated peaks were identified and qualified with reference to standard aflatoxin mixture (Romer Labs, Tulln, Austria)

2.5 Statistical evaluation of method performance

The recovery of the aflatoxins was determined by spiking their aflatoxin standards into 5 samples at the following concentration AFB1 and AFB2 (6 µg/L), and AFG1 and AFG2 (1.5 µg/L). The limit of detection (LOD) is defined as the lowest amount of analyte in a sample that can be detected (3 x noise), and the limit of qualification (LOQ) is defined as the minimum level of analysis that can reliably be quantified (10 x noise). The calculations of LOD and LOQ were done analytically by injecting 6 spiked samples. The goodness of fit of the calibration curve was measured in terms of R^2 .

Chapter 3: Results and Discussion

2.6 Chromatographic separation

Figure (3) shows the separation of AFB1, AFB2, AFG1, and AFG2 on the Agilent Zorbax Bonus C18 reversed-phase chromatographic column. The elution order was AFG2 (9.2 min), AFG1 (11.5 min), AFB2 (12.8 min), AFB1 (16.1 min), which is in agreement in order with Jalili & Jinap, (2012). The peaks were well separated and can be quantified easily.

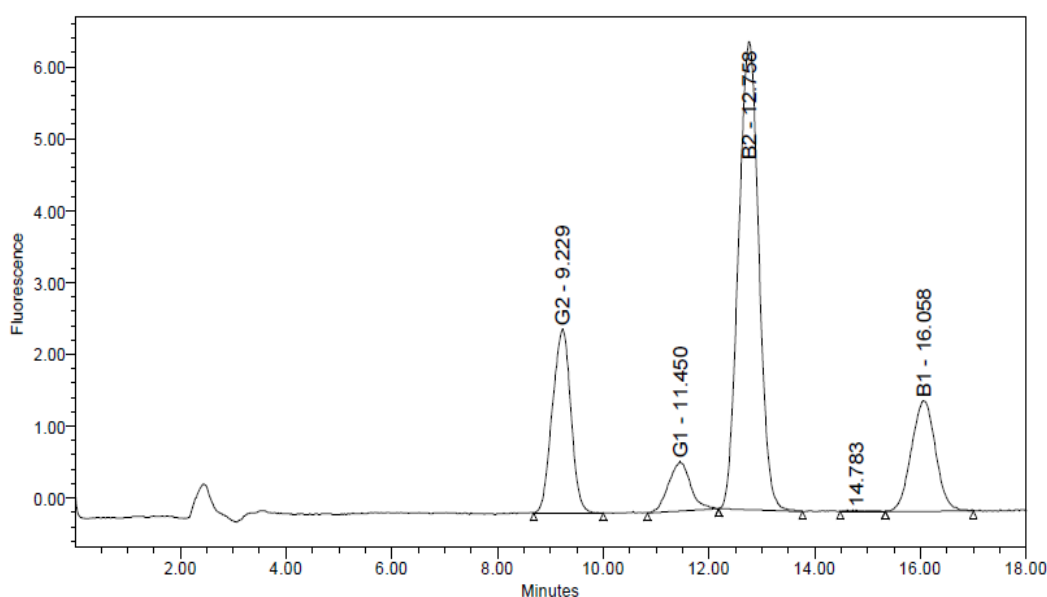


Figure 3: Chromatogram shows the peak clear

2.7 Calibration and Determination of the LOD & LOQ

The aflatoxin standards were analyzed at the ranges given in (Table 6) to establish the calibration curve (Table 7) and to determine the level of both limit of detection (LOD) and limit of quantification (LOQ) (Table 8).

The R^2 for the calibration curve of AFB1, AFB2, AFG1, and AFG2 ranged 0.995-0.997 and the variation in LOD and LOQ did not exceed 43% and

Table 6: Concentrations ($\mu\text{g/mL}$) of aflatoxin used to prepare the calibration curve

AFB1 Amt	AFB2 Amt	AFG1 Amt	AFG2 Amt
0.631	0.156	0.625	0.156
1.263	0.313	1.25	0.313
2.525	0.625	2.5	0.625
5.05	1.25	5	1.25
10.1	2.5	10	2.5

Table 7: Calibration Curves of AFB1, AFB2, AFG1, and AFG2

	Calibration Curve	R ²
AFB1	$y = 88441x - 736.7$	0.996
AFB2	$y = 21601x + 3279$	0.995
AFG1	$y = 59149x - 2953$	0.997
AFG2	$y = 91942x + 696.9$	0.996

Table 8: Determination of the LOD, LOQ for AFB1, AFB2, AFG1, and AFG2

Aflatoxin	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
AFB1	1.4 ± 2.4	4.1 ± 2.4
AFB2	6.2 ± 0.5	19 ± 0.5
AFG1	1.3 ± 2.6	3.9 ± 2.6
AFG2	7.1 ± 0.5	21 ± 0.5

n= 6

2.8 Recovery

The recovery of AFB1, AFB2, AFG1, and AFG2 was found to range between 73%-94% (Table 9).

Table 9: Calculating the recovery of the test

Peak Name	µg/mL spiked Concentration	Recovery concentration µg/mL	Recovery %
AFB1	6	4.4	73
AFB2	1.5	1.4	94
AFG1	6	4.4	73
AFG2	1.5	1.2	80

2.9 Levels of aflatoxin in the analyzed spices

Table 10: The total amount of aflatoxin in the samples

Spice	Total No. of sample	Mean total aflatoxin µg/Kg	Range µg/Kg
Chili	17	2.3 ± 2.1	0.5 - 6.7
Coriander	10	ND	ND
Fennel seeds	8	ND	ND
Paprika	6	3.1 + 6.8	0.5 – 16.9
Pepper (Black pepper)	9	1.3 ± 1.5	0.3 - 4.8
Pepper (White pepper)	8	0.5 ± 0.6	0.2 – 1.6
Turmeric	9	1.9± 2.2	0.7 – 5.4
Zataria	16	ND	ND
Mix Spices	11	0.8± 1.6	0.3 – 5.6

*ND=no detectable levels

The levels of AF contamination sample (Table 10) are considered low compared to other findings of the research conducted by Walid Hammami in Qatar “Fungal and Aflatoxin contamination of market spices” (Hammami *et al.* 2014) The

total amount of AF in black pepper detected is 84 $\mu\text{g}/\text{Kg}$, Whereas the mean of the total amount of aflatoxin in this research is $\mu\text{g}/\text{Kg}$. All the samples are below the level of LOD of AFG1, AFG2 and AFB2, and 6 sample limit of aflatoxin are higher than LOQ of AFB1.

Table 11: Levels of aflatoxin AFB1 in the analyzed spices

Type of the spice	Total No. of sample	AFB1 LOD:0.138 $\mu\text{g}/\text{mL}$, LOQ:4.14 $\mu\text{g}/\text{mL}$			
		ND	Traces	Quantified	Quantified Levels in the sample $\mu\text{g}/\text{Kg}$
chili	17	3	10	4	4.5 4.3 4.9 6.7
Coriander	10	10	0	0	
Fennel seeds	8	8	0	0	
paprika	6	3	2	1	15
Pepper (black pepper)	9	9	0	0	
Pepper (white pepper)	8	8	0	0	
turmeric	9	9	0	0	
Zataria	16	16	0	0	
mix spices	11	7	3	1	5.2
Total	94	94	15	6	

Results show that chili, paprika and mix spices were contaminated by AF AFB1 and higher than LOQ: 4.14 ($\mu\text{g}/\text{Kg}$), 4 sample of chili, one sample of mixed spices and one paprika, 4.5 $\mu\text{g}/\text{Kg}$, 4.3 $\mu\text{g}/\text{Kg}$, 4.9 $\mu\text{g}/\text{Kg}$, 6.7 $\mu\text{g}/\text{Kg}$, 5.2 $\mu\text{g}/\text{Kg}$, 15 $\mu\text{g}/\text{Kg}$ respectively, while black pepper powder, turmeric, white pepper, coriander, zataria, fennel seeds samples were undetected and less than LOQ 4.14 $\mu\text{g}/\text{mL}$. For

paprika, AFB1 levels detected in 1 out of 6 samples (16%) was above the permissible limit (Ministry of Environment and Water Decision). AFB1 occurred in 23.5% of chili and 9% of mix spices samples, but less than permissible limit (Ministry of Environment and Water Decision).

The paprika has the highest amount of aflatoxin AFB1, and that was also observed in the research of *El Mahgubi* “Distribution and toxicogenicity of *Aspergillus* section *Flavi* in spices marketed in Morocco” which proved that the highest amount of aflatoxin AFB1 was in paprika. For example, the slow air drying of the paprika and the high content of water present in the harvested fresh fruits allows the growing of mould in the post-harvest stage (Mahgubi *et al.* 2013).

Chapter 4: Conclusion

The result obtained in this study show that samples analyzed of 94 only one paprika sample contained total aflatoxin levels exceeding the permitted level of 10 μ g/Kg. AFB1 was revealed to be the dominant aflatoxin in all samples inspected in the current study. Three samples of chili, paprika, and mixed spices were found to contain AFB1 at a level of 5 μ g/Kg higher than the maximum permitted level set by the EU. These levels are considered low compared to levels obtained in other countries.

This low level of aflatoxin found in the spices collected from Abu Dhabi markets in this study may be attributed to the strict implementation of legislations and regulating rules (Regulation No (6) of 2010 Food hygiene throughout the Food Chain and Regulation No (3) of 2008. Food Traceability and Recall) at the boarder of the Emirate of Abu Dhabi.

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