UNIVERSITY OF GAZİANTEP
GRADUATE SCHOOL OF
NATURAL & APPLIED SCIENCES

DETECTION OF AFLATOXINS IN PISTACHIO NUT AND GROUND RED PEPPER SOLD IN GAZIANTEP REGION

M. Sc. THESIS
IN
FOOD ENGINEERING

BY
EBRU SET
JULY 2009

Detection of Aflatoxins in Pistachio Nut and Ground Red Pepper Sold in Gaziantep Region

M.Sc. Thesis
in
Food Engineering
University of Gaziantep

Supervisor
Prof. Dr. Osman ERKMEN

by
Ebru SET
July 2009

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NATURAL & APPLIED SCIENCES
FOOD ENGINEERING

Name of the thesis: Detection of Aflatoxins in pistachio nut and ground red pepper sold in Gaziantep Region
Name of the student: Ebru SET
Exam date: July 23, 2009

Approval of the Graduated School of Natural and Applied Sciences.

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ABSTRACT

DETECTION OF AFLATOXINS IN PISTACHIO NUT AND GROUND RED PEPPER SOLD IN GAZIANTEP REGION

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July, 2009. 106 page

In this research aflatoxin contamination of packaged and/or unpackaged ground red pepper and pistachio nuts sold in Gaziantep during 12 months period from June 2008 to May 2009 have been studied. Totally 240 ground red pepper and 240 pistachio nut samples were collected. In these samples, total aflatoxin (AFT) and aflatoxin B1 (AFB1), pH and water activity (a_w) analysis were done. Mold and yeast counts were also performed. 30.0 % (51/170) and 52.9 % (74/170) unpackaged ground red pepper samples were exceeding AFT and AFB1 legal limits (10 and 5 µg/kg) , respectively, while 1.4 % (1/70) and 5.7 % (4/70) packaged ground red pepper samples were exceeding AFT and AFB1 legal limits respectively. The highest contaminations of aflatoxins AFT and AFB1 in ground red pepper samples were seen in June. It was 97.4 ppb in AFT and 89.99 ppb in AFB1. None of the 105 (55.6 %) unpackaged and 37 (72.5 %) packaged pistachio nut samples were contaminated with aflatoxins. AFT and AFB1 were detected in 43.4 % (82/189) and 41.8 % (79/189) unpackaged pistachio nut samples, respectively, with the contamination levels ranging from 0.007 to 7.72 ppb and from 0.007 to 4.08 ppb, respectively, and only one (0.5 %) of 189 unpackaged pistachio nut sample was contained AFT and AFB1 over legal limit with contamination level 148.15 and 133.49 ppb respectively.

Aflatoxin contamination can be prevented by improving the processing methods during harvesting, drying, packaging, transportation and storage.

Keywords: Aflatoxin, ground red pepper, pistachio nut, mold

ÖZET

GAZİANTEP BÖLGESİNDE SATILAN FISTIKTA VE KIRMIZI PUL BİBERDE AFLATOKSİNLERİ BULMA

SET, Ebru
Yüksek Lisans Tezi, Gıda Mühendisliği Bölümü
Tez Yöneticisi: Prof. Dr. Osman ERKMEN
Temmuz, 2009. 106 sayfa

Bu araştırmada Gaziantepte 12 aylık dönemde Haziran 2008'den Mayıs 2009'a kadar paketli ve paketsiz satılan kırmızı pul biber ve fıstıkta aflatoksin bulaşması çalışılmıştır. Toplam 240 kırmızı pul biber ve 240 fıstık toplanmıştır. Bu örneklerde, toplam aflatoksin (AFT), aflatoksin B1, pH ve su aktivitesi (a_w) analizleri yapılmıştır. Küf ve maya sayımı da yapılmıştır. Sırasıyla % 1,4 (1/70) ve % 5,7 (4/70) paketli kırmızı pul biber örneği AFT ve AFB1 yasal limitini aşmış iken, sırasıyla, % 30,0 (51/170) ve % 52,9 (74/170) paketsiz kırmızı pul biber örneği AFT ve AFB1 yasal limitini (10 ve 5 µg/kg) aşmıştır. En yüksek aflatoksin bulaşması AFT ve AFB1, haziran ayında görülmüştür. Bu değer AFT'de 97.4 ppb ve AFB1'de 89,99 ppb dır. 105 (% 55,6) paketsiz ve 37 (% 72,5) paketli fıstık örneğinden hiç birinde aflatoksin bulaşması olmamıştır. Sırasıyla, % 43,4 (83/189) ve % 41,9 (80/189) paketsiz fıstık örneğinde bulaşma seviyesi sırasıyla, 0,007'den 7,72 ppb ve 0,007'den 4,08 ppb olan AFT ve AFB1 tespit edilmiştir ve 189 paketsiz fıstık örneğinden sadece 1 (% 0,5)'i yasal limitin üzerinde 148,15 ve 133,49 ppb AFT ve AFB1 içermektedir.

Aflatoksin bulaşması ekin toplama, kurutma, paketleme, taşıma ve depolama işlemlerinin geliştirilmesiyle önlenebilir.

Anahtar kelimeler: Aflatoksin, kırmızı pul biber, fıstık, küf

ACKNOWLEDGEMENTS

First and foremost, I express sincere appreciation to my supervisor Prof.Dr. Osman ERKMEN for his continuous encouragement, invaluable suggestions, support and guidance throughout this study.

I also would like to thank to my examining committee members, for their guidance, critical comments and suggestion regarding my thesis.

I would like to thank to my family, my husband Muammer SET and my daughter Esra Meva SET for their endless encouragement and loving supports.

I wish thank to the director of Gaziantep Province Control Laboratory Abdullah YAMAN and my staff member Veterinarian Muhittin KISA and Veterinarian Hülya ÖZDEMİR for their considerate supports.

I would like to thank Res. Asst. Aykut Önder BARAZİ and the staffs of Gaziantep University Food Engineering Department.

I also would like to thank to the University of Gaziantep Research Fount for their financial support.

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CHAPTER 1

INTRODUCTION

Fungal diseases are common place in plants and animals. In such diseases, the fungi are actively growing on and invading the body of their hosts. There is another means by which fungi can cause harm without invading our bodies. When fungi grow on a living organism or on stored food material that we consume, they may produce harmful metabolites that diffuses into their food. Mycotoxins are the secondary metabolites that fungi evolved. Mycotoxins have been reported to be carcinogenic, tremorogenic, haemorrhagic, teratogenic, and dermatitic to a wide range of organisms and to cause hepatic carcinoma in human (Wary, 1981; Refai, 1988; van Egmond, 1989).

The most recognized and intensively researched mycotoxin in the world is aflatoxin. Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus, most notably *Aspergillus flavus* and *Aspergillus parasiticus and Aspergillus nominus* (Kurtzman, Horn and Hessetline, 1987). Crops which are frequently affected include cereals (maize, sorghum, pearl millet, rice, wheat), oilseeds (peanut, soybean, sunflower, cotton), spices (chile peppers, black pepper, coriander, turmeric, ginger), and tree nuts (almond, pistachio, walnut, coconut, brazil nut). The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods. Aspergillus flavus produces B aflatoxins only, while the two other species produce both B and G ones. Toxic effects of aflatoxins include carcinogenic, mutagenic, teratogenic, and immunosuppressive activity (Eaton and Gallagher, 1994).

In Turkey, the most of the red pepper is produced in Kahramanmaraş, Gaziantep, Hatay, and Şanlıurfa in the South East region of Anatolia. About 80 % of ground red pepper was produced in Kahramanmaraş and Gaziantep regions (Hazır and Çoksoyler, 1998). In Gaziantep annual ground red pepper production was 12347.5 tones. Pistachio nut is the most important product that produced in Gaziantep. In 2008 totally 129798 tone pistachio nut was produced in Turkey. 55636.164 tones (55 %) of the pistachio nut were produced in Gaziantep (Gaziantep Agricultural Province Directorate).

As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed. The economic impact of aflatoxins derive directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health. Other adverse economic effects of aflatoxins include lower yields for food and fiber crops. Also, traditional drying methods are preferred to mechanical drying process in Turkey. Red peppers are generally dried in the sun and open air so ground red pepper made of natural dried red pepper is more likely to be contaminated with aflatoxins because of insufficient drying process. To prevent these problems the ground red peppers must be dried in a controlled environment in a certain temperature and humidity such as a factory.

The aim of this master thesis was to survey current levels of aflatoxins in ground red pepper and pistachio nut sold in Gaziantep. Samples included packaged and unpackaged ground red pepper and pistachio nut attempting to obtain a distinct pattern of contamination. Aflatoxin determination was carried out by liquid chromatography using post-column derivatization for sensitivity. Additionally, the pH, water activity and mold and yeast counting were performed.

CHAPTER 2

LITERATURE REVIEW

2.1 Fungi

Fungi are single cell living forms of life which inhabit the land, air and waters of the earth. They are everywhere. They are more highly developed than bacteria and viruses and there are many more species than are found in the microbes. It is estimated that there are over 500,000 different species. Fungi have been on earth several billion years and, quite remarkably, have had little genetic change over that period of time. They are survivalists. They can change their form from rapidly growing to no growth for thousands of years. Three groups of fungi have major practical importance: the molds, yeasts and mushrooms (Ray, 1996; Erkmen and Bozoğlu, 2007).

2.1.1 Molds

Molds include all species of microscopic fungi that grow in the form of multicellular filaments, called hyphae (which grow by elongation of their tips) (Madigan and Martinko, 2005). Some are very large and have been measured to be in the order of kilometers. In contrast, microscopic fungi that grow as single cells are called yeasts. A connected network of these tubular branching hyphae has multiple, genetically identical nuclei and is considered a single organism, referred to as a colony or in more technical terms a mycelium. Molds are reproducing by releasing tiny spores into the air. Spores lucky enough to land on moist objects may begin to grow. There are thousands of different types of mold and we encounter many of them every day, inside and out (Talaro, 1999).

Molds do not form a specific taxonomic or phylogenetic grouping, but can be found in the divisions Zygomycota, Deuteromycota, Basidomycota and Ascomycota. Although some molds cause disease or food spoilage, others are useful for their role in biodegradation or in the production of various foods, beverages, antibiotics and enzymes (Ray, 1996; Erkmen and Bozoğlu, 2007).

2.1.1.1 Biology and morphology of molds

There are thousands of known species of molds, which include opportunistic pathogens, saprotrophs, aquatic species, calders and thermophiles. Molds reproduce through small spores, which may contain a single nucleus or be multinucleate. Mold spores can be asexual (the products of mitosis) or sexual (the products of meiosis); many species can produce both types. Some can remain airborne indefinitely, and many are able to survive extremes of temperature and pressure (Ray, 1996).

Although molds grow on dead organic matter everywhere in nature, their presence is only visible to the unaided eye when mold colonies grow. A mold colony does not comprise discrete organisms, but an interconnected network of hyphae called a mycelium. Nutrients and in some cases organelles may be transported throughout the mycelium. In artificial environments like buildings, humidity and temperature are often stable enough to foster the growth of mold colonies, commonly seen as a downy or furry coating growing on food or other surfaces (Pelczar, 1993).

Mold cells are generally much larger (20-100 μm) than procaryotic cells (0.2-5 μm). The cells have rigid cell wall, do not have mucopeptide, and are composed carbohydrates. The plasma membrane contains sterol. The cytoplasm is mobile (streaming) and contains membrane bound organells (mitochondria, vacuoles). Ribosomes are 80S type and attached to the endoplasmic reticulum. The DNA is linear (chromosomes), contains histone and is enclosed in nuclear membrane. Cell division is by mitosis (asexual reproduction) and meosis (sexual reproduction). Occasionally molds such as Byssochlamys fulva and some species of Aspergillus and Penicillium are resistant to heat treatment such as pasteurization and called thermoduric (Madigan, 1997).

Molds grow on foods with cottony appearance, multinucleated, filamentation and branching. They are nonmotile. The cell wall usually composed of chitin, sometimes cellulose, and occasionally both. fungi composed of hyphae. A hypha is a branching tubular structure approximately 2-10 μm in diameter which is usually divided into cell-like units by crosswalls called septa and hyphae without cross walls are callde nonsepta hyphae. The total mass of hyphae is termed a mycelium. Hyphae can be vegetative or reproductive. Submerged hyphae digest. Absorb and distribute

nutrients from the substrate. They are also functional with absorbtion of water. (Bozoğlu and Erkmen, 2008). Later, as the mold mature, surface hyphae sprouts reproductive hyphae that produce asexual spores or sexual spores with extending in the air. Reproductive hyphae form spores either free (conidia) or in a sack. Shape, size and color of spores are used in taxonomic classification. During the asexual life cycle, the dispersed mold spores settle on a suitable and send out germ tubes that elongate into hyphae. Through continued growth and branching, an extensive mycelium is produced. A single colony of mold can easily contain 5000 spore-bearing structures (Madigan, 1997).

2.1.1.2 Mold nutrition

Molds derive energy not through photosynthesis but from the organic matter, inside of which they live. Because they lack of chlorophyll and cannot synthesize their own food the way that plant does. Typically, molds secrete hydrolytic enzymes, mainly from the hyphal tips. These enzymes degrade complex biopolymers such as starch, cellulose and lignin into simpler substances which can be absorbed by the hyphae. So, they called saprophytes. In this way, molds play a major role in causing decomposition of organic material, enabling the recycling of nutrients throughout ecosystems. Many molds also secrete mycotoxins which, together with hydrolytic enzymes, inhibit the growth of competing microorganisms. Saprophytes can also cause food spoilage and destroy wood products (Erkmen and Bozoğlu, 2008).

Some molds also form highly specialized relationship with other organisms. For example, the roots of most plants develop a mutually beneficial association with molds to form mycorrhizae. Mycorrhizae greatly increase the nutrient-absorbing capacity of the plant root. In this type of growth, the molds absorb minerals from the soil and exchange them for organic nutrients synthesized by the plant. Molds also form mutualistic associations with various animals. For example, leaf-cutting ants cut pieces of leaves and bring them into their underground nests, where they feed them to certain molds. These molds primarily live in ant nests, and the ants eat nothing but the molds. Some termites and wood-boring beetles use molds to break down the cellulose in wood, making the wood easier for the insects to digest. Plant parasites such as rusts invade plant cells via specialized structures called haustoria that absorb nutrients from the cell (Talaro, 1999).

2.1.1.3 Uses of molds

Some molds have been used as a food source. Mushrooms are a special case of mold, only existing for part of the fungus life cycle. The familiar button appearance or "fruiting" body is designed to make and release tiny spores so that the fungus can reproduce. Some spores can remain airborne indefinitely and many are able to survive extremes of temperature and pressure. Agaricus (white button) mushrooms are often found in grocery stores. Mushrooms add flavor, texture and nutritional value to many dishes (Erkmen and Bozoğlu, 2008).

Some molds, e.g., species of *Penicillium,* are useful in the preparation of Brie, Camembert, blue-veined Roquefort, and other cheeses. The *koji* molds are a group of *Aspergillus* species, notably *Aspergillus oryzae*, that have been cultured in eastern Asia for many centuries. They are used to ferment a soybean and wheat mixture to make soybean paste and soy sauce. They are also used to break down the starch in rice (saccharification) in the production of *sake* and other distilled spirits. Tempeh(*Rhizopus oligosporus*), oncom (*Neurospora sitophila*) and Quorn (*Fusarium venenatum)* are the other foods produced by using cultured molds.

Cultured molds are also useful for their role in biodegradation; many molds also produce biologically active compounds including organic acids such as citric acids, plant growth regulators such as giberellic acid. Penicillin *(Penicillium chrysogenum)* and other antibiotics (griseofulvin, cyclosporine and cephalosporin) are also obtained from molds (Ray, 1996; Erkmen and Bozoğlu, 2007).

Molds are becoming an increasingly important tool in cleaning the environment. The accumulation of pesticides and other chemicals in the environment destroys many ecosystems and placing many animal and plant species at risk. A number of molds are used in bioremediation, in which the molds are mixed with polluted water or soil, where they can decompose the organic material in pollutants to detoxify them. Molds used in this effort associating with soils are Aspergillus, Fusarium, Rhizopus, Mucor, Penicillium and Trichoderma. Molds also have been used successfully to control insects, fungus pathogens, roundworms and other organisms that cause damage and disease to agricultural crops.

Many fungi are valuable food sources for humans. Yeast, such as Saccharomyces, is an important nutritional supplement because it contains vitamins, minerals, and other nutrients.Ashbya gossypii is a producer of vitamin B2, an important nutritional supplement (Erkmen and Bozoğlu, 2008).

2.1.1.4 Mold types

Different types of mold - pathogenic mold, toxic mold, allergenic mold - are present all the time around us and in the air we breathe. In low levels, molds and mold spores are generally harmless but if their levels increase they can affect people; especially people with allergies, asthma and respiratory conditions or suppressed immune system (Ray, 1996; Erkmen and Bozoğlu, 2007).

Allergenic mold and mold spores are normally not dangerous to humans in low amounts, but they cause allergic or asthmatic symptoms.

Toxic mold is a type of mold that are know to be healthy risks to humans and animals by producing hazardous byproducts, called *mycotoxins*. Mycotoxins are fungal metabolites that have been identified as toxic agents.Mycotoxic mold and mold spores are those containing toxins in the cell wall. These types of mold can cause serious health problems in humans and animals. These molds range from short-term irritation to immunosuppression, to cancer and even death. (Talaro, 1999)

Pathogenic mold is the type of mold that causes infections. Pathogenic molds can cause serious health effects in persons with suppressed immune systems, those taking chemotherapy, and those with HIV/AIDS, or autoimmunity disorders.

2.1.1.5 Important mold genera

Molds are important spoilage microorganisms in food because they can grow under conditions in which many bacteria cannot grow, such as low pH, low water activity and high osmotic pressure. Many strains also produce mycotoxins and have been implicated in foodborne intoxication. Many are used in bioprocessing. Finally, many are used to produce food additives and enzymes.

1) *Aspergillus*

Aspergillus is the most common genus of fungi in our environment with more than 160 different species of mold. *Aspergillus* is a filamentous, cosmopolitan and ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and indoor air environment. While a teleomorphic state has been described only for some of the *Aspergillus* spp., others are accepted to be mitosporic, without any known sexual spore production. These molds appear yellow to black color on foods (Ray, 1996; Erkmen and Bozoğlu, 2007).

a) Growth and distribution

Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. In recent studies, increased levels of Reactive Oxygen Species (ROS) were shown to be correlated with increased levels of aflatoxin biosynthesis in aspergillus parasiticus. (Reverberi et al., 2008) Commonly, fungi grow on carbon-rich substrates such as monosaccharides (such as glucose) and polysaccharides (such as amylose). *Aspergillus* species are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. (Ray, 1996; Erkmen and Bozoğlu, 2007).

In addition to growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments in which there is a complete lack of key nutrients. *A. niger* is a prime example of this; it can be found growing on damp walls, as a major component of mildew (Klich, 1988).

b) Commercial importance

Species of *Aspergillus* are important medically and commercially. Some species can cause infection in humans and other animals. Some infections found in animals have been studied for years. Some species found in animals have been described as new and specific to the investigated disease and others have been known as names already in use for organisms such as saprophytes. More than 60 Aspergillus species are medically relevant pathogens (Thom, 1926). For humans there is a range of

diseases such as infection to the external ear, skin lesions, and ulcers classed as mycetomas.

Other species are important in commercial microbial fermentations. For example, alcoholic beverages such as Japanese sake are often made from rice or other starchy ingredients (like manioc), rather than from grapes or malted barley. Typical microorganisms used to make alcohol, such as yeasts of the genus Saccharomyces, cannot ferment these starches, and so *koji* mold such as *Aspergillus oryzae* is used instead (Klich, 1988). Members of the genus are also sources of natural products that can be used in the development of medications to treat human disease (US patent 6069146).

Perhaps the most well-known application of *A. niger* is as the major source of citric acid; this organism accounts for over 99% of global citric acid production, or more than 4.5 million tonnes per annum. *A. niger* is also commonly used for the production of native and foreign enzymes, including glucose oxidase and hen egg white lysozyme. In these instances, the culture is rarely grown on a solid substrate, although this is still common practice in Japan, but is more often grown as a submerged culture in a bioreactor. In this way, the most important parameters can be strictly controlled, and maximal productivity can be achieved. It also makes it far easier to separate the chemical or enzyme of importance from the medium, and is therefore far more cost-effective (Klich, 1988).

c) Aspergillosis

Some *Aspergillus* species cause serious disease in humans and animals, and can be pathogenic.

Aspergillosis is the group of diseases caused by *Aspergillus*. The most common subtype among paranasal sinus infections associated with aspergillosis is *aspergillus fumigatus.* (Bozkurt et al, 2008)

Aspergillosis is now the 2nd most common fungal infection requiring hospitalization in the United States. The symptoms include fever, cough, chest pain or breathlessness, which also occur in many other illnesses so diagnosis can be

difficult. Usually, only patients with already weakened immune systems or who suffer other lung conditions are susceptible.

In humans, the major forms of disease are:

- Allergic bronchopulmonary aspergillosis or ABPA (affects patients with symptoms that produce significant respiratory morbidity such as asthma, cystic fibrosis and sinusitis).
- Acute invasive aspergillosis (risk increases if patient has weakened immunity such as some AIDS patients and those undergoing chemotherapy).
- Disseminated invasive aspergillosis (widespread through body).

Aspergillosis of the air passages is also frequently reported in birds, and certain species of *Aspergillus* have been known to infect insects (Zirbes, 2008).

Aspergillus fumigatus: The most encountered species causing infection. It is seen abundantly in decomposing organic material, such as self-heating compost piles, since it readily grows at temperatures up to 55 ºC. People who handle contaminated material often develop hypersensitivity to the spores of *Aspergillus* and may suffer severe allergic reactions upon exposure (Finley, 1992).

Aspergillus flavus: The 2nd most encountered fungi in cases of *Aspergillus* infection. It is also known to produce the mycotoxin aflatoxin, one of the most potent carcinogens known to man. In the 1960s, 100,000 turkey poults in Great Britain died from ingesting contaminated feed. Most countries have established levels for aflatoxin in food. However, the risks associated with airborne exposure are not adequately studied and no exposure standards exist.

Aspergillus niger: The 3rd most common *Aspergillus* fungi associated with disease and the most common of any *Aspergillus* species in nature due to it's ability to grow on a wide variety of substrates. This species may cause a "fungal ball", which is a condition where the fungus actively proliferates in the human lung, forming a ball. It does so without invading the lung tissue (Erkmen and Bozoğlu, 2008).

Aspergillus clavatus: *Aspergillus glaucus* group, *Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus ustus*, and *Aspergillus versicolor* are among the other species less commonly isolated as opportunistic pathogens.

2) Other molds genera; Alternaria, Aureobasidium, Eurotium, Fusarium, Geotrichum, Monilia, Mucor, Penicilium, Rhizopus, Starchybotrys, Trichoderma, Trichthecium.

2.2 Mycotoxins

During the digestion of substrates, fungi secrete enzymes into nutrients in order to break down complex compounds into simpler compounds that can be taken up by the fungi (including mushrooms, molds, and yeasts) and used as nutrition. These digested nutrients produce secondary metabolic byproducts called mycotoxins that are released to give the fungi a competitive edge over other microorganisms and fungi (Turner, 2009).

2.2.1 Characteristics of mycotoxin producing molds

The fungi that produce mycotoxins can be divided into two categories, primary pathogens (e.g., *Coccidioides immitis* and *Histoplasma capsulatum*) and opportunistic pathogens (e.g., *Aspergillus fumigatus*, *Candida albicans*, *Candida vaginitis*, certain species of *Fusarium* and others). Primary pathogens affect otherwise healthy individuals with normal immune systems. Opportunistic pathogens produce illness by taking advantage of debilitated or immunocompromised hosts.

Molds that have been known to potentially produce these toxins are *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Stachybotrys (*Bozoğlu and Erkmen, 2008).

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. One mold species may produce many different mycotoxins and/or the same mycotoxin as another species (Robbins, et al., 2000).

The two major environmental factors associated with fungal growth are temperature and humidity. Anytime humidity is above 62%, temperature is above 80°F, and grain moisture levels are above 14% to 15%, there is a greater chance that fungi will

grow. One exception is zearalenone, which is produced under cool temperatures (less than 70°F) and moist conditions (Magan, 2004).

Molds can grow and mycotoxins can be produced preharvest or during storage, transport, processing or feeding. Mold growth and mycotoxin production are related to plant stress caused by weather extremes, to insect damage, to inadequate storage practices and to faulty feeding conditions. In general, environmental conditions, heat, water and insect damage, cause plant stress and predispose plants in the field to mycotoxin contamination. Computer models to predict mycotoxin concentrations in corn prior to harvest are based on temperature, rainfall and insect pressure (Dowd, 2004).

Because feedstuffs can be contaminated preharvest, control of additional mold growth and mycotoxin formation is dependent on storage management. After harvest, temperature, water activity and insect activity are the major factors influencing mycotoxin contamination of feedstuffs (Coulombe, 1993). Molds grow over a temperature range of 10-40°C (50-104°F), a pH range of 4-8 and above 0.7 aw (equilibrium relative humidity expressed as a decimal instead of a percentage) (Magan, 2004).

Molds can grow on feeds containing more than 12-15% moisture. In wet feeds such as silage, higher moisture levels allow mold growth if oxygen is available. Because most molds are aerobic, high moisture concentrations can exclude air and help prevent mold growth. The conditions most suitable for mold growth and for mycotoxin formation are not necessarily the same. For example, the *Fusarium* molds associated with Alimentary Toxic Aleukia have been reported to grow prolifically at 25-30°C without producing much mycotoxin, but at near freezing temperatures, they produce large quantities of mycotoxins with minimal mold growth (Joffe, 1986). Field applications of fungicides may reduce mold growth, thus reducing production of mycotoxins. However, the stress or shock of the fungicide to the mold organism may reduce mold growth and yet not reduce the production of mycotoxins (Boyacioglu et al., 1992; Gareis and Ceynowa, 1994; Simpson et al., 2001).

2.2.2 Occurance of mycotoxins

Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. Mycotoxins are produced as secondary metabolites mainly as a response to competition with other microorganisms for resources. They are not required for growth and are usually only produced during a specific period in a fungal organisms life cycle. Toxins vary greatly in their severity. Some fungi produce severe toxins only at specific levels of moisture, temperature or oxygen in the air. Some toxins are lethal, some cause identifiable diseases or health problems, some weaken the immune system without producing symptoms specific to that toxin, some act as allergens or irritants, and some have no known effect on humans. Some mycotoxins generally have more negative impacts on farm animal populations than on humans. Some mycotoxins are harmful to other microorganisms such as other fungi or even bacteria; penicillin is one example (Magan, 2004).

Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by humans, or by being used as livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and dairy products. Even temperature treatments, such as cooking and freezing, do not destroy mycotoxins (Erkmen and Bozoğlu, 2008).

Every year a significant percentage of the world's grain and oilseed crops is contaminated with hazardous mycotoxins such as aflatoxins (Phillips et al., 1994). Many biological and climatic factors influence mycotoxin contamination in agricultural commodities. Spores of molds exist in the air and soil throughout the world and can germinate when appropriate conditions arise. Mycotoxins can be formed in the field prior to and after harvest during in appropriate storage. Most specifically, presence of mycotoxin in food depends on several factors:

1) Biological: crop susceptibility and mold compatibility;

2) Enviromental: temperature, moisture, mechanical injury, insect damage, mold;

3) Harvesting: crop maturity, temperature, moisture;

4) Storage: temperature, moisture;

5) Distribution/processing: temperature, moisture; (Erkmen and Bozoğlu, 2008).

Factors that Effects the Development of Mold and Occurance of Mycotoxins;

- Humidity
- Temperature
- Kind of the food
- Chemical composition
- Climate
- Maturity of the food
- Presence of toxigenic mold
- Atmosferic oxygen
- Modified atmospheric gas
- Light
- Time
- pH

Factors that effects the development of mold and occurance of mycotoxins in pre-harvest, harvest and storage is given in Table 2.1.

2.2.3 Major groups of mycotoxins

1) Aflatoxins

Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus, most notably *Aspergillus flavus* and *Aspergillus parasiticus*, also *A. nomius and A. parasiticus*. Aflatoxins are toxic and among the most carcinogenic substances known. The native habitat of *Aspergillus* is in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and it invade all types of organic substrates whenever conditions are favorable for its growth. Conditions of high humidity and warm temperatures can raise the level of aflatoxin in foods. Favorable conditions include high moisture content (at least 7%), high temperature (33ºC), pH 5.0 and a_w 0.99 (Magan, 2004).

Table 2.1 Factors that effects the development of mold and occurance of mycotoxins

Factors	**Pre-Harvest**	**Harvest**	**Storage**
Physical			
Humidity	+	+	+
Temperature	+	+	+
Mechanical damage	+	+	+
Mixing Products	-	+	+
Chemical			
O_2	-	-	+
CO_2	-	-	+
Substrate Characteristic	+	+	+
Insects	+	-	+
Biyological			
Difference plant variants	+	-	+
Different mould strains	+	-	+
Spor load	+	+	+
Microbiological ecosystem	+	+	+
Mold infection	+	-	+

The four major aflatoxins are called B1, B2, G1, and G2 based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography. Aflatoxin B1 is the most potent natural carcinogen known (Spanos, and Gottlieb, 1976). The toxicity of aflatoxins decreases in the following order: $B_1 > M_1 > G_1 > B_2 > M_2 = G_2$. Aflatoxins M1, M2 were originally discovered in the milk of cows which fed on moldy grain. These compounds are products of a conversion process in the animals' liver. However, aflatoxin M1 is present in the fermentation broth of *Aspergillus parasiticus* (Erkmen and Bozoğlu, 2008).

High-level aflatoxin exposure produces an acute hepatic necrosis, resulting later in cirrhosis, carcinogenic and hemorrhage of the liver, altering digestive tract and

immunosupressing. Aflatoxins combine with DNA, suppressing DNA and RNA synthesis. This leads to structural changes in cell nucleoli and reduction of protein synthesis. The binding to DNA can also result in distribution of transcription, mutagenesis and carcinogenesis. Aflatoxins inhibit oxygen uptake in tissues by acting on the electron transport chain and inhibiting various enzymes and this results in decreased production of ATP. Infection with the hepatitis B during aflatoxin exposure increases the risk of hepatocellular carcinoma. The toxin can also associate with milk and milk products due to animal feed on the aflatoxin contaminated feed.

The occurence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and processing periods. Water stress, high-temperature stress, and insect damage of the host plant are major determinig factors in mold infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities, and weed competition have been associated with increased mold growth and toxin production. Aflatoxin formation is also affected by associated growth of other molds or microbes. For example, preharvest aflatoxin contamination of peanuts and corn is favored by high temperatures, prolonged drought conditions, and high insect activity; while postharvest production of aflatoxins on corn and peanuts is favored by warm temperatures and high humidity (Ray, 1996; Erkmen and Bozoğlu, 2008).

Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products are sometimes contaminated because of the animal consumption of aflatoxin contaminated feed. Crops which are frequently affected include cereals (maize, sorghum, pearl millet, rice, wheat), oilseeds (peanut, soybean, sunflower, cotton), spices (chile peppers, black pepper, coriander, turmeric, ginger), and tree nuts (almond, pistachio, walnut, coconut, brazil nut). However, the commodities with the highest risk of aflatoxin contamination are corn, peanuts, and cottonseed (Pittet, 1998; Erkmen and Bozoğlu, 2008).

Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. The FDA has established specific guidelines on acceptable levels of aflatoxins in human food and animal feed by establishing action levels that allow for the removal of violative lots from commerce. The action level for human food is 10 ppb total aflatoxins, with the exception of milk which has an action level of 0.05 ppb for aflatoxin M1. The action level for most feeds is also 10 ppb, 300 ppb for corn, beef cattle and poultry and 50 ppb for materials used to produce feed for cattle, sheep and goats. However, it is very difficult to accurately estimate aflatoxins concentration in a large quantity of material because of the variability associated with testing procedures; hence, the true aflatoxin concentration in a lot cannot be determined with 100% certainty (Finley, 1992; Eaton, 1994).

Because aflatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These include physical methods of separation, thermal inactivation, irradiation and solvent extraction, adsorption from solution, microbial inactivation, and fermentation. Chemical methods of detoxification are also practiced as a major strategy for effective detoxification:

a) Structural Degradation Following Chemical Treatment:

A diverse group of chemicals has been tested for the ability to degrade and inactivate aflatoxins. A number of these chemicals can react to destroy (or degrade) aflatoxins effectively but most are impractical or potentially unsafe because of the formation of toxic residues or the perturbation of nutrient content and the organoleptic properties of the product. Two chemical approaches to the detoxification of aflatoxins that have received considerable attention are ammoniation and reaction with sodium bisulfite.

Many studies provide evidence that chemical treatment via ammoniation may provide an effective method to detoxify aflatoxin-contaminated corn and other commodities. The mechanism for this action appears to involve hydrolysis of the lactone ring and chemical conversion of the parent compound aflatoxin B1 to numerous products that exhibit greatly decreased toxicity.

On the other hand, sodium bisulfite has been shown to react with aflatoxins (B1, G1, and M1) under various conditions of temperature, concentration, and time to form water-soluble products

.

b) Modification of Toxicity by Dietary Chemicals:

The toxicity of mycotoxins may be strongly influenced by dietary chemicals that alter the normal responses of mammalian systems to these substances. A variable array of chemical factors, including nutritional components (e.g. dietary protein and fat, vitamins, and trace elements), food and feed additives (e.g. antibiotics and preservatives), as well as other chemical factors may interact with the effects of aflatoxins in animals.

c) Alteration of Bioavailability by Aflatoxin chemisorbents:

A new approach to the detoxification of aflatoxins is the addition of inorganic sorbent materials, known as chemisorbents, such as hydrated sodium calcium aluminosilicate (HSCAS) to the diet of animals. HSCAS possesses the ability to tightly bind and immobilize aflatoxins in the gastrointestinal tract of animals, resulting in a major reduction in aflatoxin bioavailability (Heathcote, 1978; Finley, 1992; Eaton, 1994).

2) Ochratoxin

This mycotoxin is primarily produced by species of *Penicillium* and *Aspergillus*. Ochratoxin A is produced by *Penicillium verrucosum*, which is generally associated with temperate climates, and *Aspergillus* species which grow in warm humid conditions. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including cereals and their products, fruit and a wide range of beverages and spices. *Aspergillus carbonarius* is the other main species associated in warm humid conditions found mainly on vine fruit and dried vine products particularly in the Mediterranean basin (Turner, 2009). The other species that produce ochratoxin are *P.cyclopium, P.expansum* and *P. variable*. It can be damaging to the kidneys / liver, Balkan endemic nephropathy and it is carcinogen. Ochratoxin A has been shown to be weakly mutagenic, possibly by induction of oxidative DNA damage. Human exposure occurs mainly through consumption of improperly stored food products. Toxin production occurs over a wide temperature

range. Optimal conditions for ochratoxin production are at a temperature range between 68-77°F and a crop moisture content of 16% or above and a_w 0,95. Ochratoxin A has been found in maize, barley, wheat, dried cocoa beans, dried fruits, citrus fruits, peanuts, green coffee beans, corn, nuts, soybean, cheese, peppers, rye, cured meats, barley and oats, as well as in many other food products, but the occurrence of ochratoxin B is rare. It is not destroyed during the processing and cooking of food. Like most mycotoxins, ochratoxin is heat-stable. Under UV light, ochratoxins give greenish fluorescence (Erkmen and Bozoğlu, 2008).

3) Sterigmatocystin

Sterigmatocystin is a toxic metabolite structurally closely related to the aflatoxin and consists of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is mainly produced by the fungi *Aspergillus nidulans, A. versicolor, A.rugulosus, Penicilium luteum and Biopolaris* species. It can occur in moldy grain, green coffee beans, rice, oats, dried meats, dough products, nuts and cheese. It acts as an inhibitor on DNA synthesis. It causes necrosis of the liver and kidney. It is best produced at a_w 0.75. It has low oral toxicity because it is relatively insoluble in water and gastric juices. It can be detected by TLC using chloroform-methanol as a solvent system. The toxin is visualized as an orange red spot under UV light. Light yellow fluorescence develops after spraying with acetic acid. ELISA can also be used in the detection of sterigmatocystin (Erkmen and Bozoğlu, 2008).

4) Patulin

Patulin is a mycotoxin included in a group of compounds commonly known as toxic lactones. Patulin is a cyclic compound that is not fluorescent. Patulin is produced by several fungi, most of which belong to the genera *Aspergillus, Penicillium* and *Byssochylamys*. Patulin actually gets its name from the mold *Penicillium patulinum*. It is also produced by *P.expansum, Aspergillus clavatus, A.ferreus, P.cyclopium, P.claviforme* and *Bysacchlomys nivea* (Erkmen and Bozoğlu, 2008).

Patulin contamination is primarily associated with damaged and rotting fruits and fruit juices made from poor quality fruits. Patulin has been found to occur in a number of foods including apple juice, apples, peaches, grapes and pears with brown rot (Brain et al. 1956; Harwig et al. 1973), flour (Hasseltine and Graves, 1966), and

malt feed (Ukai et al. 1954). However, given the nature of the food, the manufacturing processes, or consumption practices for many foods, patulin does not appear to pose a safety concern, with the exception of apple juice (Fritz, 1981). For instance, the rotten portions of most fruits and grains typically are removed prior to consumption. In foods such as cheese, the high cysteine content of the food interacts with patulin to render it inactive (Ciegler et al., 1977). Patulin is reported to be destroyed by fermentation and thus is not found in either alcoholic fruit beverages or vinegars produced from fruit juices. Thermal processing appears to cause only moderate reductions in patulin levels, thus patulin present in apple juice will survive the pasteurization processes (IARC 1986; WHO 199; Harrison 1989; McKinley and Carlton, 1991).

It is potential carcinogen and teratogen and damage the immune system and nervous systems in animals. Patulin producing molds grow at a range of -3 to 35ºC with an optimum near 25ºC and down to a_w 0.82 (Pitt and Hocking, 1997). Patulin can be produced over the range 0-25ºC. The minimum a_w for patulin production by *P.expansum* is 0.95 at 25ºC. Patulin is produced over the narrow pH range 3.2-3.8 in apple juice. CO_2 and N_2 reduce the production of patulin. SO_2 is more effective inhibitor on molds than potassium sorbate or sodium benzoate in the production of patulin. The limit is 50 µg/kg (ppb) for this mycotoxin in both apple juice and cider. The other limits for patulin are 25 ppb in solid apple products and 10 ppb in products for infants and young children (Erkmen and Bozoğlu, 2008).

5) Citrinin

Citrinin is a mycotoxin originally isolated from Penicillium citrinum. It has since been found to be produced by a variety of other fungi *P.expansum*, *P.verrucosum* and *A.ferreus.* It is widely considered as a hazard contaminant of foods and feeds, including corn, wheat, rice, barley, rye, oat and nuts, moldy breads and cheese and is known to be cytotoxic and genotoxic to various mammalian cells. Citrinin acts as a nephrotoxin in all species in which it has been tested, but its acute toxicity varies (Bennett, 2003). It causes mycotoxic nephropathy in livestock and has been implicated as a cause of Balkan nephropathy and yellow rice fever in humans. The toxicity of citrinin was reviewed, indicating that it is a parasympathomimetic agent,

causes necrosis of tubular epithelial cells in the kidney, and in some cases, hepatotoxicity (Hanika and Carlton, 1994).

6) Trıchothecenes

The trichothecenes are a very large family of chemically related toxins produced by various species of *Fusarium (F.graminearum, F.nivea, F.oxysporum, F.sporotrichoides, F.verticilloides) Myrotecium, Trichoderma, Cephalosporium, Verticimonosporium,* and, and *Stachybotrys (S. Verticimonosporium)* (Erkmen and Bozoğlu, 2008). The tricothecenes are divided into four classes: T-1, T-2, T-3 and T-4. The T-1 mycotoxin is both rare and extremely toxic; the other three classes are most often encountered. They can be produced on ground nut, soybeans, corn, wheat, barley, oats, rye and rice (Ray, 1996; Erkmen and Bozoğlu, 2008).

It is one of the more deadly mycotoxins, if it is ingested in large amounts it can severely damage the entire digestive tract and cause rapid death due to internal hemorrhaging. It has also been implicated in human disease such as infant pulmonary hemosiderosis (Magan, 2004).

Their adversory is 1 ppm in foods indeed for human consumption. They are limited to 500 and 750 ppb for the retail and raw material stage respectively (Ray, 1996; Erkmen and Bozoğlu, 2008).

7) Zearalenon

Zearalenone (ZEA), also known as F-2 mycotoxin, is a potent estrogenic metabolite produced by some *Fusarium* species such as *F.culmorum, F.graminearium* and *F.roseum* (Erkmen, 2008). Zearalenone is heat-stable and is found worldwide in a number of high moisture cereal crops, such as maize, barley, oats, wheat, rice, and sorghum and also in bread. It can be transferred to milk and pose potential risk to humans. The formation is favored by high humidity and changing temperatures, molds grow and produce toxin. The conditions usually occur in autumn harvest and drop in the temperature during growth stimulates production of toxin. Zearalenon is a compound that survives processing and fermentation such as corn meal, beer and other fermented foods. Zeralenon producing molds usually grow in the field, causing pink discoloration of the kernel. It is partially decomposed by heat treatment but

cleaning a crop by removing its outer hull may result in higher reduction of the toxin (Magan, 2004). Zearalenone is the primary toxin causing infertility, abortion or other breeding problems, especially in swine. Zearalenone is not limited for foods. Zearalenone is a white crystalline solid. It exhibits blue-green fluorescence under longwave UV (Erkmen and Bozoğlu, 2008).

8) Deoxynivalenol

Deoxynivalenol (DON), also known as vomitoxin, is a type B trichothecene. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* and *F. culmorum*. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize, and less often in rice, sorghum, and triticale. The incidence of *Fusarium* strongly associated with moisture at the time of flowering and the timing of rainfall (Erkmen and Bozoğlu, 2008). Storage under good conditions (<14% moisture) will minimize the toxin by the toxigenic fungi.

Deoxynivalenol is thermally stable so it is difficult to eliminate from grain once formed. During the milling process of wheat it fractionates so that the higher levels concentrate in the outer bran layers and the concentration in white flour is lower than in the original grain. Because deoxynivalenol is water soluble a significant proportion can be removed by washing grain but commercially this represents an additional stage and an effluent problem. Enzymic reactions have been shown both to reduce and increase levels of deoxynivalenol (Magan, 2004). The effect of deoxynivalenol occurs through the central nervous system and is strong protein inhibitors. Irritation of the gastrointestinal tract and stomach ulceration may also occur with this mycotoxin. Deoxynivalenol has some UV absorbence so it is possible to detect it using TLC or HPLC (Erkmen and Bozoğlu, 2008).

9) Penicillic acid

A mycotoxin with antibiotic and carcinogenic activity produced by various strains of *Penıcıllıum* and *Aspergıllus*. It has produced by *P.cyclopium* (best producer), *P.puberulum and A.ochraceus*. It has been found in tobacco, sausages, corn, grains, green coffee beans, moldy market foods and cheese. It can be produced at 5ºC. It is produced in large quantities in corn with high moisture storage at low temperature, green coffee beans and apples (Erkmen and Bozoğlu, 2008).

10) Other mycotoxins

Penicillium roqueforti and *Penicillium camemberti (=Penicillium caseicola*), species used to manufacture mold-ripened cheeses, produce a number of toxic metabolites, including penicillic acid, roquefortine, isoflumigaclavines A and B, PR toxin, and cyclopiazonic acid (Scott, 1981).

Several mycotoxins induce tremors as a neurological response in farm animals; most of these fungal tremorgens contain a modified indole moiety and are produced by certain species of *Aspergillus*, *Penicillium*, and *Claviceps*. The tremorgenic mycotoxins include the penitrems, janthitrems, lolitrems, aflatrem, paxilline, paspaline, paspalicine, paspalinine, and paspalitrem A and B (Steyn et al, 1985). *Penicillium crustosum* produces penitrem A, a compound implicated in several cases of canine intoxication and one case of human tremor, vomiting, and bloody diarrhea.

Ergotism, Alternarial toxins, Cyclopiazonic acid and the yellow rice toxins (citrinin, citreoviridin, luteoskyrin, rugulosin, rubroskyrin, and related compounds) are the other toxins (Saito et al, 1971).

Fumitremorgen is produced by *Aspergillus fumigatus* and *P.lansonum* in rice. Fumonisins are produced by a number of *Fusarium* species, notably *Fusarium verticillioides* (formerly *Fusarium moniliforme = Gibberella fujikuroi)*, *Fusarium proliferatum*, and *Fusarium nygamai* in corn, rice and beans. It has hepatotoxic and carcinogenic effects (Erkmen and Bozoğlu, 2008).

2.2.4 Toxic effects of mycotoxins

The toxic effect of mycotoxins on microorganisms, plants, animals and humans health is referred to as mycotoxicosis, the severity of which depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed. Mycotoxin exposures have been linked to a variety of acute and chronic adverse health affects. Generally, these effects include acute symptoms such as pulmonary hemorrhage, dermatitis, recurring cold and flulike symptoms, burning/sore throat, headaches, excessive fatigue and diarrhea. Chronic effects include carcinogenicity, mutagenicity, teratogenicity, central nervous system effects,

immune system damage, and specific effects of the heart, liver, kidneys and other organs. (Magan, 2004). The toxic effects of mycotoxins in humans and animals may include:

- acute mycotoxicoses can cause serious and sometimes fatal diseases,
- reduced to milk and egg production by the animals subchronic exposure to mycotoxins and cause the lack of weight gain in food production,
- impairment, stimulation or suppression of immune functions and reduction resistance to infections from chronic exposures to low levels of mycotoxin,
- tumor formation, cancers and other chronic disease from prolonged exposure to very low levels of a toxin,
- mutagenic effect, capable of inducing mutations in susceptible cells of organisms,
- teratogenic effect, capable of causing deformities in developing embryos from exposure to mycotoxin,
- affecting the food supply in economic terms with mycotoxins in food-producing animals and livestock from exposure to mycotoxins (Erkmen and Bozoğlu, 2008).

2.2.5 Stability of mycotoxins in foods

Naturally occurring toxicant contamination of foods with mycotoxins is unavoidable and unpredictable and poses a unique challenge to food safety. Most mycotoxins are stable at room temperature and under neutral conditions. However, many factors must be considered during processing because temperature, pH, the presence of other constituent or enzymic action may cause reaction or breakdown to occur. Temperature and moisture content are particularly important, but these must be considered along with the other factors which may markedly affected the outcome. For example, Samarajeewa et. al. (1990) rewieved the degradetion of aflatoxins in foods under different heat treatment conditions showed losses ranging from none to almost complete destruction, while Tabata et. al. (1992) and Price and Jorgensen (1985) studied the fate of aflatoxins during cooking processes and the effects of food components on their stability.

The general trend associated with processing is to reduce the mycotoxin concentration and toxicity of the products present. However there are at least three circumstances when this may not be true. Firstly, in cereal milling, it is usually for a mycotoxin to be unequally distributed among the milling fractions, such that in some parts eg. commonly bran, a concentration of mycotoxin occur. A similar princible is applies in wet-milling processes. Secondly, chemical reaction may occur so that, while the concentration of the mycotoxins reduced, a toxic reaction product might be formed. In general this possibility has been less studied, although a hydrolyzed toxic product has been reported from fumonisin (Hopkins and Murphy, 1993) and toxic product from citrinin (Trivedi et al., 1993). Thirdly, the presence of molds in the commodity may result in further mycotoxin formation during processing under specific conditions.

Aflatoxins are toxic mold metabolites produced by toxigenic strains of *Aspergillus* species. Primary commodities susceptible to aflatoxin contamination include corn, peanuts and cottonseed and animal-derived foods such as milk when the animal is fed aflatoxin-contaminated feed. Risks associated with aflatoxin-contaminated foods can be reduced through the use of specific processing and decontamination procedures. Factors, which influence the effectiveness of a specific process or procedure, include the chemical stability of the mycotoxin, nature of the process, type and interaction with the food/feed matrix and interaction with multiple mycotoxins if present. Practical decontamination procedures must:

(1) inactivate, destroy, or remove the toxin,
(2) not produce or leave toxic residues in the food/feed,
(3) retain the nutritive value of the food/feed,
(4) not alter the acceptability or the technological properties of the product, and, if possible,
(5) destroy fungal spores.

2.2.6 Prevention and Controlling of Mycotoxins

Every year a significant percentage of the world's grain and oilseed crops is contaminated with hazardous mycotoxins such as aflatoxins (Phillips et al., 1994). Control of mycotoxin producing fungi and mycotoxin contamination in foods and

feeds has been proved difficult. Many biological and climatic factors influence mycotoxin contamination in agricultural commodities and these factors are difficult to control. Detection, removal and diversion are reasonable means for preventing the entry of mycotoxins into the food chains (Figure 2.1). The best way of controlling mycotoxin contamination is by prevention and can be accomplished by reducing fungal infection in growing crops through the adoption of suitable cultural practices, by rapid drying or by the use of suitable preservatives (Smith and Moss, 1985; Sinha, 1993). If contamination can not be prevented, a way to either remove or destroy the toxin will allow consumption of the commodities with reduced adverse effect (Krogh, 1987). Physical, chemical and biological methods have been investigated in order to prevent the growth of mycotoxin producing fungi, eliminate or reduce the toxin levels, degrade or detoxify the toxins in foods and feeds. Mycotoxins can be eliminated or detoxified by physical, chemical or biological techniques. Many chemicals including numerous acids, alkalis, aldehydes, oxidizing agents and several gases have been tested for their ability to degrade or inactivate aflatoxin and many other mycotoxins (Smith and Moss, 1985; Samarajeenwa et al., 1990; Thanaboripat, 2002). Most of the monitoring for mycotoxins in foods have focused on aflatoxins.

Chemical treatment by ammoniation has been found to be an effective method to detoxify aflatoxin-contaminated corn and other commodities. Sunflower meal, an excellent source of protein supplement in poultry and animal feeds in Pakistan has also been tested for aflatoxin detoxification by ammoniation (Ahmad et al., 1995). Butylated hydroxyanisole (BHA), a phenolic antioxidant, has been reported to inhibit the growth of toxigenic species of *Aspergillus, Fusarium*, and *Penicillium* (Thompson, 1996).

The application of salt for controlling *A. flavus* in peanut was investigated. The result indicated that low concentrations of sodium chloride stimulated aflatoxin production whereas high concentrations inhibited fungal growth and aflatoxin production (Thanaboripat et al., 1992). High concentrations of sodium chloride may adversely affect the water activity required for growth and toxin production or it may be that sodium ions interfere with ion transport in the organism.

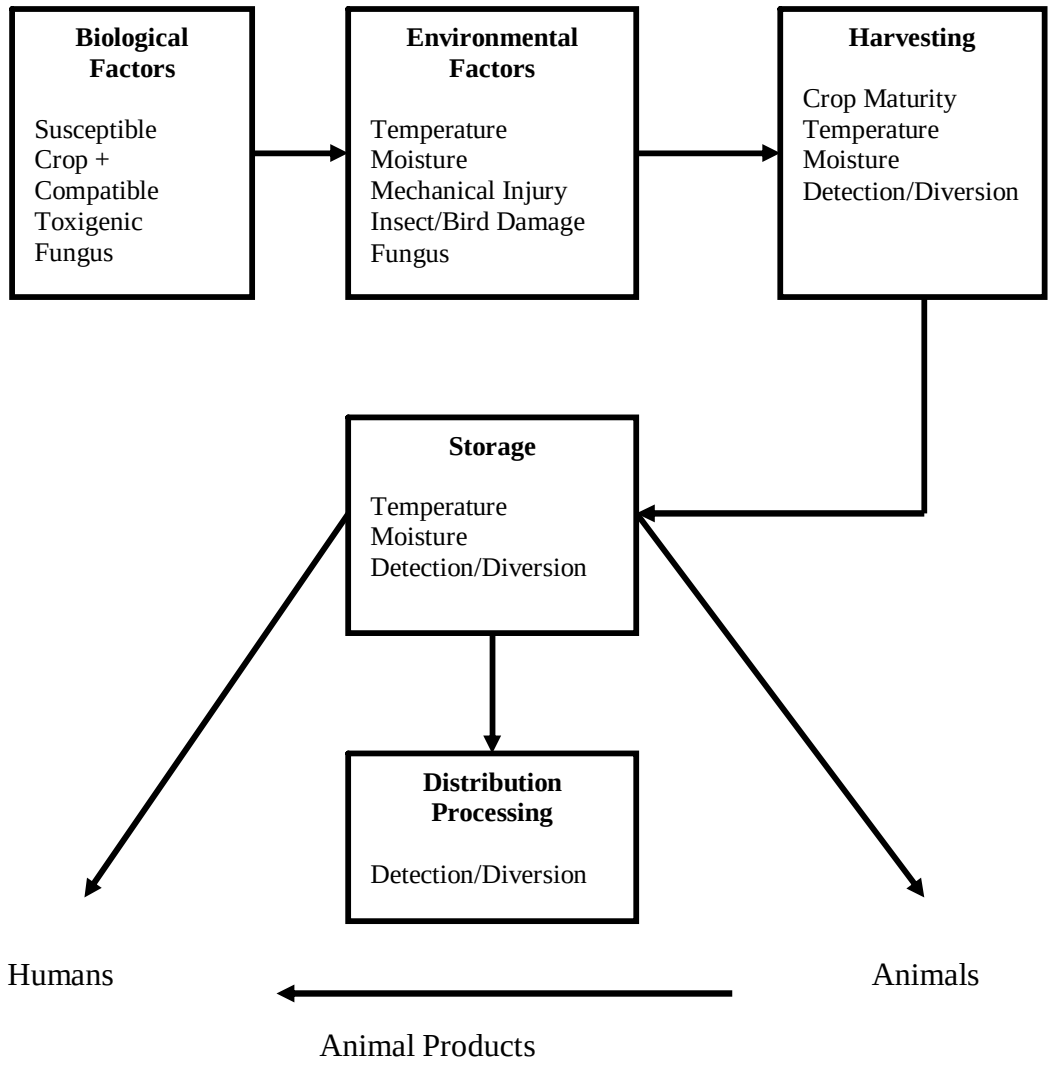


Figure 2.1. Factors influencing mycotoxin occurrence in the food chain (Pestka and Casale, 1988).

Natural compounds from plants have been used traditionally to preserve foods in countries like Japan, India and Russia (Wilson and Wisniewski, 1992). The extracts of some local plants show the ability to suppress the growth of toxigenic fungi and hence, the toxin production. Essential oils of cinnamon, peppermint, basil, origanum, the flavoring herb Epazote, clove, and thyme caused a total inhibition of *A. flavus* on maize kernels (Montes-Belmont and Carvajal, 1998). Natural plant extracts may provide an alternative way to protect foods or feeds from fungal contamination (Yin and Cheng, 1998). While dealing with grain protection, fumigation is the preferred method for applying substances into the bulks in order to control the biotic factors which damage the grains (Paster et al., 1995).

Various investigators have reported that a number of microorganisms affected the production of aflatoxin in a competitive environment. A mixture of Lactobacillus species has been reported to reduce mold growth and inhibit aflatoxin production by *A. flavus subspp. parasiticus* (Gourama and Bullerman, 1995). Rhizopus oligosporus, a fungus used in the preparation of tempeh, was reported to inhibit the growth of *A. flavus* and *A. parasiticus* and also aflatoxin (Ko, 1978; Thanaboripat et al., 1996). *Ganoderma* is a medicinal fungus and has been treasured for this value in China for more than two thousand years (Liu, 1993). *Ganoderma lucidum* (Lingzhi mushroom) can be produced in large quantities by solid state fermentation and submerged fermentation. Effect of mycelial growth of Ling Zhi mushroom on the growth and aflatoxin of *Aspergillus parasiticus* was studied. When growing *G. lucidum* as mycelium on sorghum seeds for 3 days or more before inoculating spores of *A. parasiticus*, the results showed that aflatoxin production was inhibited (Thanaboripat et al., 2002).

Trichoderma species have been reported to inhibit fungal pathogen growth and development (Elad et al., 1983). The ability of these antagonists to attack fungal pathogens has led to the use of Trichoderma spp. as potential biocontrol agents. Possible antagonism by Trichoderma have been suggested to involve antibiotics and/or enzyme production, as well as parasitism (Elad et al., 1983; Benhamou and Chet, 1993). *Trichoderma viride* and *T. harzianum* have been reported to inhibit the growth of *A. flavus* and *F. moniliforme* (Calistru et al., 1997). However, mycoparasitism is not the mechanism involved in the inhibitory interaction of either *A. flavus* or *F. moniliforme* with Trichoderma spp.

It has been realized that mycotoxins are very important because the contamination of mycotoxins pose serious problems in public health, agricultural and economic aspects. Prevention is still the best method for preventing mycotoxin production. Thus, all efforts have to be made in order to prevent the mold growth and mycotoxin production along the entire food chain.

2.3 Studıes About Aflatoxıns

The AFTs were first heavily researched and understood after the death of more than 100,000 young turkeys on poultry farms in England (turkey disease) that were found to be related to the consumption of Brazilian peanut meal (Bennett and Klich, 2003). AFTs are carcinogenic to humans and are classed as dangerous food contaminants (Butler and Barnes, 1963). AFT B1 is the most potent natural carcinogen known, and is usually the major AFT produced by toxigenic strains.

Aflatoxins (AFTs) are widely distributed toxins produced by strains of *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* (Kurtzman et al., 1987). *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins (Creppy, 2002).
AFTs may contaminate a number of granular foods, including cereals, grains and groundnuts. The incidence of AFTs and their concentration in contaminated products depend on the conditions of temperature and humidity during crop growth and storage (Ellis et al., 1991).

Paprika and chili powders are one of the most important spices, widely cultivated and used all over the world. Also they have considerable economic importance for most of the countries. Whereas high quality products are desired, major disadvantages are implicated with conventional spice processing (Buckenhu, 2003). However, chillies are susceptible to contamination by aflatoxins or mycotoxins produced following the growth of certain fungal species, especially, *Aspergillus flavus* (Paterson, 2007; Romagnoli et al., 2007; O' Riordan and Wilkinson, 2008). Aflatoxins are carcinogenic to humans and are classed as dangerous food contaminants (Butler and Barnes, 1963).

In the case of spices such as chillies, recently introduced European Union (EU) regulations allow the presence of 5 µg/kg of aflatoxin B1 and 10 µg/kg of total aflatoxins (Paterson, 2007; Romagnoli et al., 2007).

From the mycological perspective, there are qualitative and quantitative differences in the toxigenic abilities displayed by different strains within each aflatoxigenic species. For example, only about half of *A. flavus* strains produce AFT-producing species more than 10^6 µg/kg (Klich et al., 1988).

Juan et al. (2008) reported that dried fruits and nuts commercialized in Rabat could be contaminated with AFs. Theses authors analyzed 100 samples of pistachio, peanuts, dried raisins, figs and walnuts for the presence of AFs. Results from this study showed a weak contamination of peanut (only one positive sample out of 20).

Several environmental factors are known to influence aflatoxin production, but temperature and relative humidity are considered to be the most critical. Studies performed on hazelnuts and pistachios suggested that optimum temperature and relative humidity for aflatoxin production is 25–30ºC and 97–99%, respectively (Diener and Davis, 1966, 1967; Schindler et al., 1967; Northolt et al., 1976; Simsek et al., 2002). Additional factors are water activity, moisture content and substrate composition (Sakai et al., 1984).

Contamination in pistachio nuts to various degrees has been reported by Hadavi (2005) where the early-split nuts were the most contaminated, growth split nuts less contaminated, and pistachios with sound hulls being almost clean. Abdulkadar et al., (2002) reported that the highest incidence and levels of aflatoxin contamination was in pistachio without shell followed by pistachio with shell. Finally, when the aflatoxin level in various foods are assessed our finding are consistent with studies by Selim et al. (1996) on common Egyptian foods which indicate high prevalence of aflatoxin B1 in nuts and seeds (82%) followed by spices (40%) and cereal grains (21%).

2.4 Productıon Process Of Pepper

A few varieties of pepper fruit (Capsicum annuum) are extensively employed as spices after drying and grinding. In general it is called paprika in worldwide. In Turkey, the most of the red pepper were produced in Kahramanmaraş, Gaziantep, Hatay and Şanlıurfa in the South East region of Anatolia. About 80 % of ground red pepper was produced in Kahramanmaraş and Gaziantep regions (Hazır and Çoksoyler, 1998). In Gaziantep the annual ground red pepper production was 12347.5 tone in 2008 (Gaziantep Agriculture Province Directorate).

2.4.1 Harvesting

Harvesting at the correct stage of maturity is essential to produce high quality peppercorns. The pepper spikes are picked when one or two of the berries on the spike begin to turn orange and the berries are hard to touch. The whole spikes of berries are picked by hand. The flavour and pungency of pepper develop as the berries ripen and mature. Correct harvesting techniques could be said to be the most important factor in the production of a high quality final product.

2.4.2 Sorting

After harvest the pepper berries are removed from the stems either by hand as seen in Figure 2.2 or by beating with sticks or by using a minim mechanical thresher. The stems are separated out and discarded.

Figure 2.2 Vilagers sorting pepper berries

2.4.3 Drying

This is by far the most important section in the process. The inability to adequately dry the produce will, at the very least, slow down the whole process and possibly lead to mold growth. Any pepper with even a trace of mold cannot be used for processing.

Traditionally, pepper berries are spread on floor to dry using the natural heat from the sun. The best drying surfaces to use are bamboo mats coated with fenugreek paste, concrete floors or high density black polythene, which give a better quality and cleaner final product. The berries should be raked several times a day to turn them over and allow them to dry fully. Sun drying takes anything from 7 to 10 days depending upon the local climate and the density of the pile of berries. In our country whole peppers are dried in the open air and exposed to sunlight, which usually takes 8–10 days (Doymaz and Pala, 2002). (Figure 2.3) Many of the fungal species that are encountered in dried pepper arise from this drying method and aflatoxins are produced.

Nowadays, driers are used to dry the pepper. Industrially it is faster, hygenic and safe method to dry peppers. In this way aflatoxin contamination is reduced.

Figure 2.3 Pepper berries are dried on flor

2.4.4 Grading

Pepper is graded by size, colour and relative density. Colour grading is done by hand. Small machines are available for grading pepper according to the size or relative density of the peppercorns.

2.4.5 Grinding

Grinding may also add value but must be done carefully as there are difficulties. A whole, intact product can be easily assessed for quality whereas a ground product is more difficult. There are basically two types of grinders - manual grinders and mechanical grinders.

Manual grinding mills are generally for the small-scale processor. Mechanical grinding mills suitable for peppercorns include horizontal and vertical plate mills and hammer mills. In Figure 2.4 end product can be seen which is ready to packaging.

Figure 2.4 Ground red pepper ready for packaging

2.4.6 Packaging

Packaging material; Packaging of pepper especially if it is ground requires polypropylene. Polythene can not be used as the flavour components diffuse through it.

Simple sealing; the bags can be sealed simply by folding the polypropylene over a hacksaw blade and drawing it slowly over the flame of a candle. However, this extremely uncomfortable as the hacksaw blade heats up and can burn the hands of the operator. However, this is a very common technique.

Sealing machines; a sealing machine will speed this operation up considerably and produce a much tidier finish (which is very important).

2.4.7 Storage

Dried peppers must be stored in moisture-proof containers away from direct sunlight. The optimal conditions for a store are a low temperature, a low humidity and free from pests. The store should be located in a shaded, dry place. Strong smelling foods, detergents and paints should not be stored in the same room. To prevent rodents entering, the roof should be completely sealed.

2.5 Productıon Process Of Pıstachıo

The pistachio is one of the favorite tree nuts of the world and is widely cultivated in saline, dry and hot areas of Middle East, Mediterranean countries and USA. It contributes substantially to the agricultural exports of some of these countries. Several species of the genus Pistacia are reffered to as pistachio, but only the fruits of Pistachio vera attain sufficiently large size to be acceptable to consumers as edible nuts. (Maskan, 1997). In Figure 2.5 Pistachio nuts on the tree can be seen.

Figure 2.5 Pistachio nuts on the tree

As harvested pistachios are charged to the processing line, in dry processing they pass through the following steps:

• Dehulling
• Separation of trash
• Drying
• Splitting
• Salting and roasting

2.5.1 Dehulling

In some countries the nuts are usually dehulled very soon after harvest and the nuts in shell are then stored and processed (fast lane). In Turkey, however, the nuts are stored in-hull, sometimes for many months, or even for years (slow lane). Early de-hulling has the advantage of avoiding staining of the shell, but has the disadvantage of exposing the split nuts at an early stage to Aspergillus flavus and A. parasiticus spores which have the potential to produce aflatoxin. Dehulling can either be carried out using a wet-process or a dry process (Arrus, 2005).

2.5.2 Trash Removal

Most of the hulls, bunches and leaves are separated from the pistachios by the peeler and are conveyed out of the area. Some very small remaining pieces of hulls, bunches and leaves are separated at this stage. Trash removal is also a dry procedure. Trash separation is also used in the wet hulling lines, after pistachios are peeled by the wet peeler.

2.5.3 Drying

In order to prevent spoilage, the pistachio nuts must be dried immediately after harvest. The advantage of driying and storing in hull is the lessened possibility of insect damage and relief of the work load at harvest time. Due to that pistachio nuts are dried with forced air at 60 ºC in dryers. The moisture content of freshly harvested nuts, with up to 45 % is reduced to 5 % in about 6 to 7 hours or by sun drying for 3 to 4 day (Woodroof, 1967; Yonniotis, 1996).

2.5.4 Split

The next step is to sort the split from the unsplit nuts. Most years, only a little more than half of the total nuts are naturally split. In Turkey unsplit pistachios are splitting by hand.

2.5.5 Salting and Roasting

The nuts are salted and roasted in shell. The pistachio nuts are salted by treatment with a salt solution (approximately 15 %), then dried in a rotary-screen drier at 25 ºC for 1 hr until the moisture taken up by the salting is removed the tempğerature is than raised to 65 ºC over a 30 min period and held for 20 min, then cooled and packaged (Woodroof, 1967; Yonniotis, 1996).

2.6 Mycotoxin Regulations

Since the discovery of the aflatoxins in 1960, regulations have been established in many countries to protect consumers from the harmful effects of mycotoxins that may contaminate foodstuffs, as well as to ensure fair practices in food trade. Various factors play a role in decision-making processes focused on setting limits for mycotoxins. These include scientific factors to assess risk (such as the availability of toxicological data), food consumption data and detailed knowledge about possibilities for sampling and analysis, and socio-economic issues (Egmond et al., 2007).

In Table 2.2 A comparison between the limits proposed by the Turkey for mycotoxin regulation in foods and the European regulations EC No. 1881/2006 (European Commission, 2007) and limits in Turkey (Turkish Food Codex, Regulation No:2008/26) is given.

Table 2.2 A comparison between the limits proposed by the Turkey for mycotoxin regulation in foods and the European regulations EC No. 1881/2006 (European Commission, 2007) and limits in Turkey (Turkish Food Codex, Regulation No:2008/26).

Mycotoxins	Commodity	Max. Levels (µg/kg) in Turkey	Max. Levels (µg/kg) in European
AFB1	Cereals and cereal products	2	2
	Baby foods including cereals	0.1	0.1
	Spices	5	5
	Nuts and dried fruits	5	2
AFs	Cereals and cereal products	4	4
	Spices	10	10
	Nuts and dried fruits	5	2
AFM1	Liquid milk	0.05	0.05
	Baby milk	0.025	0.025
OTA	Wine	2	2
	Cereals	5	5
	Cereal products	3	3
	Dried raisins	10	10
	Soluble coffee	10	10
	Cereals products for babies	0.5	0.5
ZEA	Cereals	100	100
	Corn	350	200
	Bread	50	50
	Cereal products for babies	20	20

Table 2.1 (continue)

Fumonisins (B1+B2)	Unprocessed maize	4000	4000
	Maize and maize-based foods intended for direct human consumption	1000	1000
	Maize-based breakfast cereals and maize-based snacks	800	800
	Processed maize-based foods and baby foods for infants and young children	200	400
PAT	Fruit juice	50	50
	Baby foods	10	10
DON	Unprocessed cereals other than durum wheat, oats and maize	1250	1250
	Unprocessed durum wheat and oats and maize	1750	1750
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	750	750
	Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	500
	Processed cereal-based foods and baby foods for infants and young children	200	200

AFB1: Aflatoxin B1
AFT: Total Aflatoxin
AFM1: Aflatoxin M1
OTA: Ochratoxin A
ZEA: Zearelanon
PAT: Patulin
DON: Deoksinivalenol

CHAPTER 3

MATERIAL AND METHODS

3.1. Material and Reagents

3.1.1. Materials

The materials and equipments used in this thesis were;

- High Performans Liquid Chromotography (HPLC, DIONEX, California Avenue, Palo Alto, CA 94304 U.S.A) in total aflatoxin (AFT) and aflatoxin B1 (AFB1) analysis components are:
  - Dionex P680 HPLC Pump,
  - Dionex ASI-100 Automated Sample Injector,
  - Dionex RF 2000 Fluorescence detector (FLD),
  - Dionex Thermostatted Column Compartment TCC-100,
  - Computer (Packard Bell) and software (Chromeleon) and
  - HPLC column (C18 -250 mm-5µm- 4.6 mm).
- In post-column derivatization Kobra Cell was used.
- Analytical Balance (0.01 g scaled, Sartorious, Goettingen)
- Blender (1000 ml, Waring Commercial Blender, Torrington)
- Pure Water System (Elga, Lane End Industrial Park High Wycombe, HP14 3BY, UK)
- Ultra Pure Water System (Elga, Purelab Option Q)
- Filter Paper (Whatman No:4 with pore size 30µm, rough filter papers)
- Glass Microfiber Filter Paper (Whatman, pore size 1.6µm)
- Vorteks (Nüve, Istanbul)
- Rotronic hygolab (Rotronic HygroLab 3 set; 160 E. main street, Huntington, NY 11743, USA)
- İmmunoaffinity Column (R-Biopharm Rhone, Glasgow, Scotland)
- pH-meter (WTW pH/mV/Temperature Meters, Models 720; 3150 Commercial Ave Northbrook, IL 60062, USA)

3.1.2 Reagents

HPLC solvents (grade acetonitrile, methanol and technical methanol) were purchased from Interlab (Adana, Turkey). Ultrapure water was obtained from an Elga Purelab Option Q apparatus from Elga (Lane End Industrial Park High Wycombe Bucks HP14 3BY UK). The immunoaffinity columns were supplied by Sincer (Izmir, Turkey). Aflatoxin B1, B2, G1 and G2 mix standard was provided by Supelco (Sincer, Izmir Turkey) and stock solutions (50 µg/ml) were prepared in methanol and stored at 1ºC. Stock solution mix containing the four aflatoxins B1, B2, G1 and G2 with concentrations of 0.3 µg/ml and 1 µg/ml, were diluted in methanol and stored at 21ºC. The other reagents were;

- Standard stock solutions of aflatoxins B1, B2, G1 and G2 in methanol (0.3-1µg/kg) and was purchased from Sincer (Ziya Gökalp Bul. 17/4 Alsancak, Izmir) Supelco (Bellefonte, PA, USA).
- Sodium chloride (J.T Baker Deventer, Nederland)
- Potassium bromide (KBr, Merck, Darmstadt Germany)
- Acetonitrile (LC grade, Sigma-Aldrich, Steinheim Germany)
- Methanol (LC grade, Sigma-Aldrich, Steinheim Germany)
- Methanol (Technical grade, Sigma-Aldrich, Steinheim Germany)
- Extraction solvent for pepper; Methanol-water (8:2 v/v)
- Extraction solvent for pistachio; Methanol-water (8:2 v/v)
- Nitric acid (HNO_3) 4M (Merck, Darmstadt Germany)
- Mobil phase solvent was prepared by water, acetonitrile, methanol which were used 5:2:3 in volume. For each liter of mobile phase 350 µl 4M Nitric acid and 120 mg potassium bromide was added and mixed to dissolve.
- Potato Dextrose Agar (PDA, Difco, Voigt Global Distribution Inc P.O.Box 1130 Lawrence, KS 66044-8130 U.S.A)

3.2 Sample Collection

240 ground red pepper and 240 Gaziantep pistachio nuts were purchased from the markets, supermarkets, groceries and local retails in Gaziantep, Turkey. They were packaged (about 500 grams) or unpackaged (about 250 grams) samples which were represent the different places, qualities and brands on sale in Gaziantep. Samples

were placed in black plastic bags and taken to the laboratories with in 1 hour and the samples were protected from sunlight during the transfer. Aflatoxin analysis carried out in the Gaziantep Province Control Laboratory and the pH and a_w analysis carried out in the Gaziantep University Department of Food Engineering. The samples were stored at 4ºC prior to the analysis. Two sample was taken and two parallel analyses were performed for each sample. Average result of the four analyse was given as a result.

3.3 Analyses of Ground Red Pepper

3.3.1 Aflatoxin Analyses

3.3.1.1 Sample preparation

Pepper samples were taken to the laboratory at room temperature approximately 18±5ºC and stored at 4ºC. Samples were protected from sunlight. Because Aflatoxin is deteriorate under sunlight.

3.3.1.2 Aflatoxin analyses in ground red pepper

50 gram of test sample was added 5 g NaCl and 200 ml extraction solvent (methanol: water 8:2 v/v) in an erlenmeyer. After shaking 30 min, the sample extract was filtered from rough and whatman no:4. 10 ml filtrate was diluted with 40 ml ultra pure water in a measuring cylinder (100 ml). After filtration of the sample with glass micro fiber filter paper, 10 ml of filtrate was passed from the immunoaffinity column at a speed of 2-3 ml/min. After that, the column was washed with 10 ml of distilled water. Finally, bound aflatoxins were eluted slowly with 1 ml methanol and pushed air through the column to collect the last drops of elute in 5ml amber vial and dilute with 1 ml water. The extract was taken to a 1.8 ml vial before the injection (AOAC 2000).

3.3.1.3 HPLC

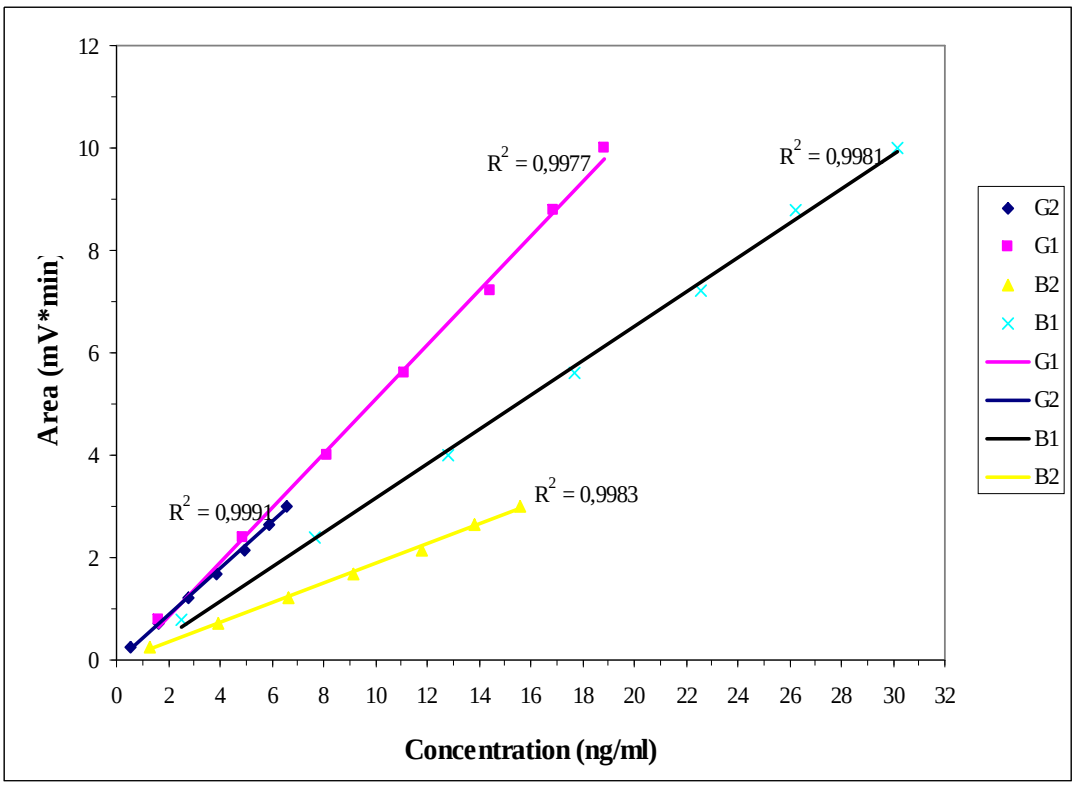
Samples were analyzed for aflatoxin by HPLC method as indicated in the Association of Official Analytical Chemists (AOAC Official Method, 2000). The presence of aflatoxins was detected by HPLC using a post-column derivatization electrochemically generated bromine (Kobra cell) and a fluorescence detector at 360 nm (excitation) and 440 nm (emission). The HPLC column was Supelco and the

flow rate was 1ml/min. 100 µl sample was injected to HPLC automatically and the sample was read for 15 min. The peaks in chromatogram were evaluated according to the standard curve. Example of sample peak for AFT and AFB1 chromotogram is given in appendix figure 1.

3.3.1.4 Preparation of standard curve

In this study 7 point calibration curve was used. Seven different concentrations (as shown in Table 3.1) prepared from the stock standard (which has a known concentration of 2600ng/ml) and a curve was drawn. (concentration of aflatoxin (ng/ml) against peak area (mV*min). The peaks obtained were then compared with sample peaks obtained with that of aflatoxin standards.

Table 3.1 Calibration curves of Aflatoxins B1,B2,G1 and G2



3.3.2 Chemical analyses

3.3.2.1 pH analyses

10 g ground red pepper sample was diluted with 90 ml diluted water, then mixed and pH was measured with a pH-meter.

3.3.2.2 Water activity analyses

Approximately 30 g ground red pepper sample was put into the cup of Rotronic hygolab at room temperature and then the small cup holder was placed into the probe (AW-DIO Probe) of Rotronic hygrolab device to measure a_w. After a_w equilibrium was remained constant, result was read from device and recorded as a_w of the sample.

3.4 Analyses of Pistachio Nut

3.4.1 Aflatoxin analyses

3.4.1.1 Sample preparation

Pistachio samples were taken to the laboratory at room temperature approximately 18±5ºC and stored at 4ºC. Samples were protected from sunlight; because Aflatoxins are undergo to light degradation. All samples were finely grinded and thoroughly mixed since homogenized sample is also very important.

3.4.1.2 Aflatoxin analyses

50 grams of test portion was blended with 5 g NaCl and 250ml extraction solvent (methanol: water 3:2 v/v) in a blender. It was blended 3 min with high speed and the extract was filtered from rough filter and whatman no: 4. 20 ml filtrate was diluted with 20 ml ultra pure water in a measuring cylinder (100 ml). After filtered the sample from glass micro fiber filter paper, 10 ml of filtrate was passed from the immunoaffinity column at a speed of 2-3 ml/min. After that, the column was washed with 10 ml of distilled water. Finally, bound aflatoxins were eluted slowly with 1 ml methanol and pushing air through the column to collect the last drops of elute in 5 ml amber vial and dilute with 1 ml water. The extract was taken to a 1.8 ml vial before the injection (AOAC 2000).

3.4.1.3 HPLC

Samples were analyzed by HPLC method as explained in the Association of Official Analytical Chemists (AOAC Official Method, 2000). The presence of aflatoxins was detected by HPLC using a post-column derivatization electrochemically generated bromine (Kobra cell) and a fluorescence detector at 360 nm (excitation) and 440 nm (emission). The HPLC column was Supelco and the flow rate was 1 ml/min.

3.4.1.4 Preparation of standard curve

Standard curve was prepared as disscuss in 3.3.1.4 and the calibration curve was seen in Table 3.1.

3.4.2 Chemical analyses

3.4.2.1 pH analyses

10 g pistachio nut sample was diluted with 90 ml diluted water, then mixed and pH was measured by a pH-meter.

3.4.2.2 Water activity analyses

Approximately 30 g pistachio nut sample was put into the cup of Rotronic hygolab and then the small cup holder was placed into the probe (AW-DIO Probe) of Rotronic hygrolab device to measure aw. After a_w equilibrium was remained constant, aw amount was read from device and it was recorded as a_w of the sample.

3.5 Mold and Yeast Count in Ground Red Pepper and Pistachio Nut

3.5.1 Preparation of solutions, media and homogeneity of sample

For mold and yeast count, potato dextrose agar was prepared. Agar media was sterilized in autoclave at 121ºC for 15-20 min. Then, the pH of media was adjusted with addition of tartaric acid. And they were poured into sterile petri dishes (Erkmen and Bozoglu, 2007).

Peptone water was used for sample and dilution waters. Peptone was readily powdered for mixing with distilled water and it was weighed on sensitive balance. 0.1 % peptone water was used during preparation of dilution waters, culture solution and preparation of sample waters. 1 g of peptone powder was mixed with 1000 ml of distilled water in a balloon, then distributed to bottles and test tubes in required amount and sterilized at 121ºC for 20 min in autoclave (Erkmen and Bozoglu, 2007).

Dilution water was used for diluting the sample of food products that was expected to include microorganisms in it. In order to prepare sterile dilution water, first 9 ml of 0.1 % peptone water was put into test tubes in aseptic conditions and mouths of

tubes were closed by the lids. After preparing 9 ml of 0.1 % peptone water, test tubes were sterilized in autoclave at 121ºC for 20 min and stored at refrigerator for further use (Erkmen and Bozoglu, 2007).

About 25 g of sample (either ground red pepper and pistachio nut) were added into 225 ml 0.1 % sterile peptone water and mixed thoroughly for 5 min and allowed to stand on table for to precipitate solids. This homogenization gives 10^{-1} dilution and further necessary dilutions were prepared using 9 ml 0.1% sterile peptone water (Erkmen and Bozoglu, 2007).

Mold and Yeast counts were done by spread plate technique using PDA. After drying of surface of the inoculated plates, they were incubated at 25 ºC for 72 h in incubator. After incubation, formed colonies on PDA were counted from plates containing colonies between 30 and 300 (Erkmen and Bozoglu, 2007).

3.6 Statistical Analyses

The results of AFT and AFB1 for packaged and unpackaged ground red pepper and pistachio nut samples were compared by one-way analysis of variance (one-way ANOVA) to test the significant differences at α=0.05 level. Differences among sample means were reported to be significant when $P<0.05$ and they were reported to be nonsignificant when $P>0.05$. Microsoft Office Excel 2003 (Microsoft corporation, U.S.A) was used in the calculations.

Safety

Aflatoxin are carcinogens and care should be exercised to avoid personal exposure and potential risk of contamination. All handling of pure compounds were done in the fume hood with protective gear such as safety glasses, gloves, laboratory coat and a disposable face mask. The glasswares were washed with hypochlorite and dilute acid before re-using and the waste materials treated with hypochlorite before disposal.

CHAPTER 4

RESULTS AND DISCUSSION

In this study 240 ground red pepper and 240 pistachio nut samples (each about 250g) were collected during 12 months period from June 2008 to May 2009. In these samples, total aflatoxins (AFT) and aflatoxin B1 (AFB1), pH and water activity (a_w) analysis were done. Mold and yeast counts were also performed in this study. The results of aflatoxin were evaluated for 3 months with respect to AFT and AFB1 levels over and below of legal limits for unpackaged and packaged samples. Therefore the results for 12 months were given in four periods. The temperature and relative humidity of the periods were; 1. period 24.4°C and 66.5 %, 2. period 9.6°C and 80 %, 3. period 19°C and 70.9 % and 4. period 35.3°C and 55.8 % respectively.

4.1 Ground Red Pepper

4.1.1 Aflatoxin analysis

Levels of AFT and AFB1 contamination in 240 different ground red pepper samples collected during 12 months were detected by HPLC method. The legal limits of AFT and AFB1 for ground red pepper are the same in Turkey (Turkish Food Codex, Regulation No:2008/26) and European Union (Commission Regulation, EC No. 1881/2006) maximum allowed levels are 10 ppb (μg/kg) and 5 ppb (μg/kg) for AFT and AFB1 respectively. The contamination levels of AFT and AFB1 for ground red pepper for 12 months are given in Appendix A.

The number (percent) of unpackaged and packaged ground red pepper samples contaminated with AFT and AFB1 for first period (September, October and November 2008) is given in Table 4.1. In this period, 75.7 % (28/37) of unpackaged and 100 % (23/23) of packaged ground red pepper samples were below legal limits of AFT. For AFB1, 78.4 % (29/37) of unpackaged and 100 % of packaged ground red pepper samples were below legal limits and 21.6 % (8/37) of unpackaged ground red pepper samples were over legal limits.

Table 4.1. Number of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in September, October and November 2008

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds and Yeasts	
	< 10 ppb	> 10 ppb	< 5 ppb	> 5 ppb	$< 10^5$	$> 10^5$
Unpackaged (n=37)	28 (75.7 %)	9 (24.3 %)	29 (78.4 %)	8 (21.6 %)	34 (91.9%)	3 (8.1%)
Packaged (n=23)	23 (100 %)	-	23 (100 %)	-	23 (100 %)	-
Total (n=60)	51 (85.0 %)	9 (15.0 %)	52 (86.7 %)	8 (13.3 %)	57 (95 %)	3 (5 %)

In September, 28.6 % (4/14) and 35.7 % (5/14) of unpackaged ground red pepper samples were exceeding AFT and AFB1 legal limits respectively. They were 30.8 % (4/13) for unpackaged and packaged in October. There was no unpackaged or packaged ground red pepper sample over legal limits in November. The contamination levels of AFT and AFB1 in unpackaged ground red pepper samples were changed from 0.133 to 57.3 ppb and 0.066 to 55.9 ppb, respectively, from September to November. They were changed from 0.08 to 2.06 ppb and 0.08 to 1.95 ppb, respectively, in packaged ground red pepper samples. The statistical results were given in Appendix D1 and Appendix D6 with respect to the unpackaged and packaged samples. There are nonsignificant difference ($P>0.05$) between these 3 months unpackaged ground red pepper samples with respect to the AFT and AFB1. There are significant difference ($P<0.05$) between these 3 months packaged ground red pepper samples, respectively.

The number (percent) of unpackaged and packaged ground red pepper samples contaminated with AFT and AFB1 for second period (December 2008, and January and February 2009) is given in Table 4.2. In this period, 11.1 % (5/45) and 13.3 % (11/45) of unpackaged ground red pepper samples were over the legal limits of AFT and AFB1 respectively. None of the packaged ground red pepper sample was exceeding the legal limit of AFT and only one 6.7 % of packaged sample was exceeding the legal limit of AFB1.

Table 4.2. Number of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in December 2008 and January and February 2009

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds and Yeasts	
	< 10 ppb	> 10 ppb	< 5 ppb	> 5 ppb	$< 10^5$	$> 10^5$
Unpackaged (n=45)	40 (88.9 %)	5 (11.1 %)	34 (48.3 %)	11 (13.3 %)	43 (95.6%)	2 (4.4 %)
Packaged (n=15)	15 (100 %)	-	14 (93.3 %)	1 (6.7 %)	15 (100 %)	-
Total (n=60)	55 (91.7 %)	5 (8.3 %)	48 (80.0 %)	12 (20.0 %)	58 (96.7%)	2 (3.3%)

In December, 7.7 % (1/13) and 38.5 % (5/13) of unpackaged ground red pepper samples were exceeding AFT and AFB1 legal limits respectively. They were 11.1 % (2/18) in January for both AFT and AFB1 and 14.3 % (2/14) and 28.6 % (4/14) in February, respectively. The contamination levels of AFT in unpackaged ground red pepper samples were changed, from 0.69 to 10.38 ppb, 0.087 to 19.96 ppb and from 0.1 to 12.26 ppb in December, January and February respectively while they were changed from 0.61 to 7.63 ppb, from 0.067 to 18.82 ppb and from 0.08 to9.0 ppb, for AFB1 for three respective months. The contamination levels of AFT in packaged ground red pepper samples were changed from 0.09 to 0.69 ppb, 0.21 to 9.32 ppb and from 0.36 to 1.16 ppb for three respective months while AFB1 changed from 0.09 to 0.58 ppb, from 0.14 to 6.86 ppb and from 0.32 to 1.00 ppb for three respective months. The statistical results were given in Appendix D2 and Appendix D7 with respect to the unpackaged and packaged samples. There are nonsignificant difference ($P>0.05$) between these 3 months unpackaged ground red pepper samples with respect to the AFT and AFB1. There are significant difference ($P<0.05$) between these 3 months packaged ground red pepper samples, respectively.

The number (percent) of unpackaged and packaged ground red pepper samples contaminated with AFT and AFB1 for third period (March, April and May 2009) is given in Table 4.3. In this period, 72.1 % (31/43) of unpackaged and 100 % (17/17) of packaged ground red pepper samples were below legal limits of AFT and 27.9 % (12/43) of unpackaged ground red pepper samples were over legal limits. For AFB1, 51.1 % (22/43) of unpackaged and 5.9 % (1/17) of packaged ground red pepper samples were over legal limits.

Table 4.3. Number of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in March, April and May 2009

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds and Yeasts	
	< 10 ppb	> 10 ppb	< 5 ppb	> 5 ppb	$< 10^5$	$> 10^5$
Unpackaged (n=43)	31 (72.1 %)	12 (27.9 %)	21 (48.9 %)	22 (51.1 %)	35 (81.4%)	8 (18.6%)
Packaged (n=17)	17 (100 %)	-	16 (94.1 %)	1 (5.9 %)	17 (100 %)	-
Total (n=60)	48 (80.0 %)	12 (20.0 %)	37 (61.7 %)	23 (48.3 %)	52 (86.7%)	8 (13.3%)

In March, 33.3 % (5/15) unpackaged and no packaged ground red pepper samples were exceeding AFT legal limits. They were 53.3 % (8/15) and 20.0 % (1/5) for AFB1 for unpackaged and packaged ground red pepper samples respectively. In April, 23.1 % (3/13) and 69.2 % (9/13) of unpackaged ground red pepper samples were exceeding legal limits of AFT and AFB1 respectively. In May, AFT and AFB1 exceeding the legal limits with 26.7 % (4/15) and 33.3 % (5/15) of unpackaged ground red pepper samples, respectively,. The contamination levels of AFB1 in unpackaged ground red pepper samples were changed from 0.45 to 18.71 ppb, from 1.25. to 15.37 ppb and from 0.25 to 33.49 ppb in March, April and May respectively while they were from 0.51 to 20.03 ppb, from 1.31 to 17.86 ppb and from 0.35 to 36.49 ppb for AFT. The contamination levels of AFT in packaged ground red pepper samples were changed from 0.06 to 8.9 ppb, from 0.19 to 4.76 ppb and from 1.18 to 2.73 ppb for respective three moths while they were 0.06 to 8.25, 0.12 to 1.98 and 1.06 to 2.67 for AFB1 respectively. The statistical results were given in Appendix D3 and Appendix D8 with respect to the unpackaged and packaged samples. There are nonsignificant difference ($P>0.05$) between these 3 months unpackaged ground red pepper samples with respect to the AFT and AFB1. There are nonsignificant difference ($P>0.05$) between these 3 months packaged ground red pepper samples, respectively.

The number (percent) of unpackaged and packaged ground red pepper samples contaminated with AFT and AFB1 for last period (June, July and August 2009) is given in Table 4.4. In this period, 55.6 % (25/45) of unpackaged and 6.7 % (1/15) of packaged ground red pepper samples were exceeding legal limits of AFT while 44.4

% (20/45) of unpackaged and 93.3 % (14/15) packaged red ground red pepper samples were below legal limits of AFT. For AFB1, 73.3 % (33/45) of unpackaged and 13.3 % (2/15) of packaged ground red pepper samples were exceeding legal limits while 26.7 % (12/45) of unpackaged and 86.7 % (13/15) of packaged ground red pepper samples were below legal limits of AFB1.

Table 4.4. Number of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in June 2009 and July and August 2008

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds and Yeasts	
	< 10 ppb	> 10 ppb	< 5 ppb	> 5 ppb	$< 10^5$	$> 10^5$
Unpackaged (n=45)	20 (44.4 %)	25 (55.6 %)	12 (26.7 %)	33 (73.3 %)	32 (71.1%)	13 (28.9%)
Packaged (n=15)	14 (93.3 %)	1 (6.7 %)	13 (86.7 %)	2 (13.3 %)	15 (100%)	-
Total (n=60)	34 (56.7 %)	26 (43.3 %)	25 (41.7 %)	35 (58.3 %)	47 (78.3%)	13 (21.7%)

The highest contaminations of AFT and AFB1 in ground red pepper samples were seen in this period. The contamination levels of AFT in unpackaged ground red pepper samples were changed from 0.52 to 97.4 ppb, from 2.04 to 69.84 ppb and from 1.61 to 65.8 ppb in June, July and August respectively. The contamination levels of AFB1 in unpackaged ground red pepper samples were changed from 0.52 to 89.99 ppb, from 1.98 to 64.72 ppb and from 1.37 to 62.42 ppb, for respective three months. In June, 42.9 % (6/14) and 50 % (7/14) of unpackaged ground red pepper samples were exceeding AFT and AFB1 legal limits respectively. They were 81.3 % (13/16) and 93.8 % (15/16), respectively, in July, and 37.5 % (6/16) and 68.8 % (11/16), respectively, in August. The contamination levels of AFT in packaged ground red pepper samples were changed from 1.24 to 17.54 ppb, from 0.15 to 1.6 ppb and from 0.38 to 2.04 ppb in these respective three months where samples were changed from 0.79 to 15.46 ppb, from 0.15 to 1.1 ppb and from 0.28 to 1.92 ppb for AFB1. The statistical results were given in Appendix D4 and Appendix D9 with respect to the unpackaged and packaged samples. There are nonsignificant difference ($P>0.05$) between these 3 months. unpackaged ground red pepper samples with respect to the AFT and AFB1. There are nonsignificant difference ($P>0.05$) between these 3 months packaged ground red pepper samples, respectively.

In this study, 240 ground red pepper samples were collected from June 2008 to May 2009 (12 months) from different markets, supermarkets and groceries that were sold in Gaziantep (Table 4.5). 30.0 % (51/170) and 43.5 % (74/170) unpackaged ground red pepper samples were exceeding AFT and AFB1 legal limits, respectively, while 1.4 % (1/70) and 5.7 % (4/70) packaged ground red pepper samples were exceeding AFT and AFB1 legal limits respectively. The contamination level of AFT and AFB1 with unpackaged ground red pepper samples were ranged from 0.087 ppb to 97.4 ppb and from 0.067 ppb to 89.99 ppb, respectively. On the other hand, the packaged ground red pepper samples were contaminated in the range of 0.06 ppb to 17.54 ppb and 0.06 ppb to 15.46 ppb with respect to the AFT and AFB1. The statistical results were given in Appendix D5 and Appendix D10 with respect to the unpackaged and packaged samples. There are significant difference ($P<0.05$) between the four periods of unpackaged ground red pepper for AFT and AFB1. There are nonsignificant difference ($P>0.05$) between the four periods of unpackaged and packaged ground red pepper for AFT and AFB1.

Table 4.5. Number of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected from June 2008 to May 2009 (12 months)

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds and Yeasts	
	< 10 ppb	> 10 ppb	< 5 ppb	> 5 ppb	$< 10^5$	$> 10^5$
Unpackaged (n=170)	119 (70.0 %)	51 (30.0 %)	96 (56.5 %)	74 (43.5 %)	144 (84.7%)	26 (15.3%)
Packaged (n=70)	69 (98.6 %)	1 (1.4 %)	66 (94.3 %)	4 (5.7 %)	70 (100%)	-
Total (n=240)	188 (78.3 %)	52 (21.7 %)	162 (67.5 %)	78 (32.5 %)	214 (89.2%)	26 (10.8%)

4.1.2 Mold and yeast count

Mold and Yeast count was evaluated according to the legal limit which was 10^5 in Turkish Food Codex (Microbiological Criteria). Results were given in Table 4.1 to 4.5 for three months period. In the first period 8.1 % unpackaged ground red pepper sample was exceeding the legal limit while none of the packaged sample was exceeding the legal limit. In the second period 4.4 % unpackaged ground red pepper sample was exceeding the legal limit while none of the packaged sample was exceeding the legal limit. In the third period 18.6 % ground red pepper sample was

exceeding the legal limit while none of the packaged sample was exceeding the legal limit. In the last period 28.9 % unpackaged ground red pepper sample was exceeding the legal limit while none of the packaged sample was exceeding the legal limit. In total 10.8 % ground red pepper sample was over the legal limit where 89.2 % sample was below the legal limit. No relation seen between the mold and yeast count and aflatoxin levels.

4.1.3 Chemical Analysis

pH analyses were carried out in the ground red pepper samples during 12 months period from June 2008 to May 2009. The results of pH analyses for ground red pepper samples were given in Table 4.6 The results of first period (September, October and November 2008) given in Table 4.6 It is changed from 4.13 to 5.62. 43 % (26/60) sample values were greater than 5.0. In the second period (December 2008 and January and February 2009), results were ranged from 4.42 to 5.44. 32 % (19/60) sample values were greater than 5.0. In the third period (March, April and May 2009), results were ranged from 4.32 to 5.98. 36.7 % (22/60) sample values were greater than 5.0. In the last period (June 2009 and July and August 2008), results were ranged from 4.11 to 5.87. 32 % (19/60) sample values were greater than 5.0.

Table 4.6. Results of pH analyses in packaged and unpackaged ground red pepper samples in first and second periods

pH range	1. period		2. period	
	Unpackaged (n=37)	Packaged (n=23)	Unpackaged (n=45)	Packaged (n=15)
pH < 5.0	19 (51.4 %)	15 (65.2 %)	30 (66.7 %)	11 (73.3 %)
pH >5.0	18 (48.6 %)	8 (37.8 %)	15 (33.3 %)	4 (26.7 %)

	3. period		4. period	
	Unpackaged (n=43)	Packaged (n=17)	Unpackaged (n=45)	Packaged (n=15)
pH < 5.0	26 (60.5 %)	12 (70.6 %)	29 (64.4 %)	12 (80 %)
pH >5.0	17 (39.5 %)	5 (29.4 %)	16 (35.6 %)	3 (20 %)

Water activity (a_w) analyses were also carried out in the ground red pepper samples during 12 months period from June 2008 to May 2009. The results were given in Table 4.7. In the first period (September, October and November 2008) results were ranged from 0.25 to 0.78. In the second period (December 2008 and January and February 2009), results were ranged 0.37 to 0.67. In the third period (March, April and May 2009), results were ranged from 0.34 to 0.67. In the last period (June 2009 and July and August 2008), results were ranged from 0.40 to 0.62.

Table 4.7. Results of water activity (a_w) analyses in packaged and unpackaged ground red pepper samples for the four period

a_w range	1. period		2. period	
	Unpackaged (n=37)	Packaged (n=23)	Unpackaged (n=45)	Packaged (n=15)
0.79-0.60	11 (29.7 %)	9 (39.13 %)	15 (33.3 %)	2 (3.3 %)
0.59-0.40	13 (35.15 %)	3 (13.04 %)	29 (64.4 %)	13 (86.7 %)
0.39-0.20	13 (35.15 %)	11 (47.83 %)	1 (2.3 %)	-

	3. period		4. period	
	Unpackaged (n=43)	Packaged (n=17)	Unpackaged (n=45)	Packaged (n=15)
0.79-0.60	15 (34.9 %)	5 (29.4 %)	6 (13.3 %)	2 (13.3 %)
0.59-0.40	26 (60.5 %)	9 (52.9 %)	38 (84.4 %)	13 (86.7 %)
0.39-0.20	2 (4.6 %)	3 (17.7 %)	1 (2.3 %)	-

In Turkey, Aydin et al. (2007) reported high levels of AFB1 contained in red pepper powder samples in the level up to 40.9 ppb. Erdogan (2004) tested 44 red-scaled pepper and 28 powdered red pepper samples obtained from Erzurum and found AFT in 8 (18.2 %) red-scaled pepper samples, aflatoxin contamination was ranged from 1.1 to 97.5 ppb. and 3 (10.7 %) red pepper powder samples contaminated with aflatoxin ranged from 1.8 to 16.4 ppb. He reported that 5 (11.4 %) red-scaled pepper and one (3.6 %) red pepper powder samples were exceeding the legal limit. Ağaoğlu (1999) analyzed the powdered unpackaged red pepper samples collected from Van, the contamination level of AFB1 was ranged from 1.1 to 44.0 ppb and indicated that

57.5 % (23/40) samples were exceeding AFB1 legal limit. Yildirim et al. (1997) found aflatoxin in 23.5 % (8/34) red pepper samples collected from Bursa and Sakarya, and the levels of AFB1 changed between 1.6 and 15 ppb. Dokuzle (2001) were analyzed the ground red pepper samples collected from Bursa and the level of aflatoxin contamination ranged from 5 to 25 ppb and 43.3 % (13/30) ground red pepper samples had AFB1 over legal limits. Hazir and Coksoyler (1998) reported that 32.6 % (46/141) ground red pepper samples collected from different regions of Turkey were contaminated with AFB1 in the range of 0.45-80.25 ppb. Taydaş and Aşkın reported that 65.3 % (83/127) red pepper samples were contaminated with AFB1 from 1.25 to 28.50 ppb. Kanbur et al. (2006) analyzed the 50 red peppers samples for aflatoxins sold in Kayseri. They reported that red pepper samples contaminated with AFB1 from 1.5 to 70.1 ppb and 6.0 % (3/50) red pepper had higher level of AFB1 over legal limits. The number of red pepper samples sold in different region of Turkey contaminated with AFT over legal limits was reported by Ministry of Agriculture (1996) with 46.7 % samples, by Aydın et al. (2005) with 12.0 % samples and by Kanbur et al. (2006) with 6.0 % samples. According to these results, red pepper sold in Turkey contained the AFT and AFB1 levels from 1.1 to 97.5 ppb and from 0.45 to 80.25 ppb respectively. In our study, the contamination levels of AFT and AFB1 were ranged from 0.087 to 97.4 and 0.067 to 89.99 ppb, respectively, for unpackaged ground red pepper samples. On the other hand, the contamination level of AFT and AFB1 ranged from 0.06 to 17.54 ppb and from 0.06 to 15.46 ppb, respectively, for packaged ground red pepper samples. 30 % (51/170) and 43.5 % (74/170) of unpackaged and 1.4 % (1/70) and 5.7 % (4/70) packaged ground red pepper samples were exceeding the legal limit in AFT and AFB1, respectively.

Fazekas et al. (2005) analyzed red pepper samples for aflatoxin contents and found 25.7 % (18/70) ground red pepper samples contained AFB1 in Hungary. Abdulkadar et al. (2004) reported that 66.7 % (4/6) chilli pepper powder was contained AFT over legal limits with the contamination level ranging from 5.60 to 69.28 ppb, in Qatar. Romagnoli et al. (2007) found aflatoxins contamination in red pepper (45.5 %) in the range of 0.57-30.7 ppb in Emilia Romagna Region, Italy. In Moroco, from Rabat and Sale, black pepper were analyzed for AFB1 and the levels of AFB1 were about 0.09 ppb (Zinedine et al., 2006). In Korea, 17.1 % (7/41) red pepper powder

samples and 13.3 % (2/15) red pepper paste samples contained aflatoxins (Cho et al., 2008) where the samples were collected from ten cities including Gangneung, Wonju, Seoul, Anyang, Daejeon, Cheongju, Gwangju, Sunchang, Daegu, Busan. In Bahrain, Manama, 3 (50 %) of 6 red chilli pepper contained AFT with 35.9, 52.6 and 69.2 ppb, respectively, (Musaiger et al., 2007).

In Turkey most of the ground red pepper sunlight dried as intact on soil and then they were sold with and/or without packaging but most of the ground red pepper samples have been consuming without packaging. This region climate (temperature and relative humidity) can be available for mold growth and survival of spores in soil and air. Therefore mold spores from soil and air easily contaminate with ground red pepper during sunlight drying by intact soil. This mold contamination can produce aflatoxin since this drying period is long to allow mold growth and aflatoxin production and after drying ground red peppers were stored in open sack to allow further mold spore contamination and growth of mold spores. The detection of aflatoxins in unpackaged ground red pepper in higher number of samples indicated that aflatoxin formation is a big problem for the ground red pepper produced by sunlight drying and storage during selling. The fresh dried whole ground red pepper and powdered red pepper were produced in September in Turkey and then consumed through one year period. The numbers of unpackaged ground red pepper contaminating with aflatoxins exceeding legal limits were higher in June, July and August indicated the contamination of molds and formation of aflatoxins during one year period under suitable environmental conditions for growth of molds. Since level of AFT and AFB1 over legal limits in unpackaged ground red pepper samples in the order; 24.3 and 21.6 % for first period (September, October and November), 11.1 and 13.3 % for second period (December, January and February), 27.9 and 51.1 % for third period (March, April and May) and 55.6 and 73.3 % for fourth period (June, July and August). Since the new products get out in September, our value in the first period is greater than the second period because the samples taken in the first period was not completely the new product.

This study is the first report on the co-occurrence of AFT and AFB1 for ground red pepper sold in Gaziantep and higher amount of ground red pepper producing region. The results obtained in this research confirm that ground red pepper could be

affected by mycotoxin contamination due to the climatic conditions, especially temperature of the region, in agreement with previous surveys held among others (Aydin et al., 2007, Erdogan, 2004; Ağaoğlu, 1999; Yildirim et al., 1997).
The incidence of aflatoxins in food is relatively high in tropical and subtropical regions, where the warm and humid weather provides optimal conditions for the growth of molds (Rustom, 1997). Gaziantep is in the southeast of Turkey. It has warm and humid weather (especially in fall, winter and spring) in where molds easily grow.

Technology used in the ground red pepper production is very important for the contamination and growth of molds, and production of aflatoxins. Traditionally, whole ground red peppers are dried in the open air and exposed to sunlight by intact soil, which usually takes 8-10 days (Maskan, 1997; Doymaz and Pala, 2002). It is a common method, but it has several disadvantages such as being time consuming, prone to contamination with dust, soil and sand particles, and insects depending on weather. Many of the mold species that are encountered in ground red pepper arises from this drying conditions and aflatoxins are produced (Inan et al., 2007). To prevent these problems the ground red peppers must be dried in a controlled environment in a certain temperature and humidity such as in a factory. Unpackaging and storage is another processing problem for mold contamination and aflatoxin formation. Since in this study, the unpackaged ground red peppers had more aflatoxins than the packaged ground red peppers. Packaging would prevent the mold spore contamination and restricts of air to prevent the mold growth. Storage temperature, relative humidity (RH), composition of gases in packages and composition of product are important parameters in the growth of molds and formation of aflatoxin (Sarimehmetoglu et al., 2004; Erkmen and Bozoglu, 2008). Aflatoxins are mycotoxins produced by molds *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, which grow on harvested crops, during drying and storage (Erdogan, 2004; Erkmen and Bozoglu, 2008). Ground red pepper is a very sensitive product for aflatoxin formation depending on unsuitable processing conditions (Coksoyler, 1999). Many survey showed the presence of xerophilic mold species, especially *Aspergillus fumigatus, A. flavus, A. niger* in most ground red pepper samples (Seenappa and Kempton, 1980; Mathyastha and Bhat, 1984;

Delcourt et al., 1994; El-Kady et al., 1995; Adegoke et al., 1996; Freire et al., 2000; Vrabcheva, 2000).

The possibility of presence of aflatoxins in ground red pepper is high due to growing in the climate with high RH, raining and warm temperature (Williams et al., 2004, Kanbur et al., 2006). The ground red pepper has high exporting capacity due to higher amount of production in Turkey. But the presence of aflatoxins in ground red pepper can have a risk on public health beside economical lost. The strict measures are necessary to produce high quality ground red pepper. These measures can avoid contact with soil during drying and prevent contamination during processing and storage from air, prevent mold growth with low RH and unsuitable temperature. In addition, packaging is also a good measure to supply attractive and healthy products in hygienic condition. There are great variations between the lowest and highest limits of AFT and AFB1 contents of ground red peppers. The presence of aflatoxins with highest amount in ground red pepper would be due to unfavorable harvesting and production techniques, production under unsanitary conditions, temperate climates, storage conditions after production and during selling and the use of soiled ground red pepper in the production of scaled ground red pepper. The cities producing ground red pepper with high amount in Southern region of Turkey have Mediterranean climate conditions with hot and dried air in summer, and cool and rainy (moist) in fall, winter and spring. Our results showed that the level of aflatoxins increased from fall to summer. The production of ground red pepper using modern technology (such as drying, low temperature and humidity) with the prevention and elimination of mold contamination, storage of ground red pepper under favorable conditions (low RH) and packaging after production can prevent mold growth and aflatoxins formation.

4.2. Pistachio Nut

4.2.1. Aflatoxin analyses

The occurrence of AFT and AFB1 in pistachio nut collected in Gaziantep during 12 months period (from June 2008 to May 2009) was surveyed and 240 pistachio nut samples were analyzed for AFT and AFB1 by HPLC methods. The legal limits of AFT and AFB1 in pistachio nut can’t be greater than 10 ppb for AFT and 5 ppb for AFB1 according to Turkey standards (Turkish Food Codex, Regulation

No:2008/26). The contamination levels of AFT and AFB1 for Gaziantep pistachio nut for 12 months are given in Appendix B.

The number (percent) of unpackaged and packaged pistachio nut samples contaminated with AFT and AFB1 for first period (September, October and November 2008) is given in Table 4.8. 60 samples were analyzed, both of AFT and AFB1 were detected in 70.6 % (36/51) unpackaged pistachio nut samples with different contamination levels ranged from 0.01 to 6.44 ppb and from 0.01 to 1.68 ppb, respectively. Both of AFT and AFB1 were detected 77.8 % (7/9) in packaged pistachio nut samples with different contamination levels ranged from 0.01 to 0.07 ppb and from 0.01 to 0.03 ppb, respectively. The statistical results were given in Appendix E1 and Appendix E6 with respect to the unpackaged and packaged samples. There are significant difference ($P<0.05$) between these 3 months unpackaged pistachio nut samples with respect to the AFT and AFB1. There are nonsignificant difference ($P>0.05$) between these 3 months packaged pistachio nut samples, respectively.

Table 4.8. Number (percent) of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in September, October and November 2008

Type of samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds	
	<10 ppb	ND	<5 ppb	ND	$< 10^4$	$> 10^4$
Unpack. (n=51)	36 (70.6 %)	15 (29.4 %)	36 (70.6 %)	15 (29.4 %)	51 (100%)	-
Packaged (n=9)	7 (77.8 %)	2 (22.2 %)	7 (77.8 %)	2 (22.2 %)	9 (100%)	-
Total (n=60)	43 (71.7 %)	17 (28.3 %)	43 (71.7 %)	17 (28.3 %)	60 (100%)	-

ND: Not Detected

The number (percent) of unpackaged and packaged pistachio nut samples contaminated with AFT and AFB1 for second period (December 2008, January and February 2009) is given in Table 4.9. 60 samples were analyzed, both of AFT and AFB1 were detected in 27.3 % (12/40) unpackaged pistachio nut samples with contamination level ranged from 0.045 to 7.72 ppb and from 0.04 to 4.08 ppb, respectively. Both of AFT and AFB1 were detected in 25.0 % (4/16) packaged pistachio nut samples with different contamination level ranged from 0.019 to 0.41 ppb and from 0.019 to 0.36 ppb, respectively. The statistical results were given in

Appendix E2 and Appendix E7 with respect to the unpackaged and packaged samples. There are nonsignificant difference (P>0.05) between these 3 months unpackaged pistachio nut samples with respect to the AFT and AFB1. There are nonsignificant difference (P>0.05) between these 3 months packaged pistachio nut samples, respectively.

Table 4.9. Number of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in December 2008, January and February 2009

Type of pistachio nut samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds	
	<10 ppb	ND	<5 ppb	ND	$< 10^4$	$> 10^4$
Unpack. (n=44)	12 (27.3 %)	32 (72.7 %)	12 (27.3 %)	32 (72.7 %)	43 (97.7%)	1 (2.3%)
Packaged (n=16)	4 (25.0 %)	12 (75.0 %)	4 (25.0 %)	12 (75.0 %)	16 (100%)	-
Total (n=60)	16 (26.7 %)	44 (73.3 %)	16 (26.7 %)	44 (73.3 %)	59 (98.3%)	1 (1.7%)

The number (percent) of unpackaged and packaged pistachio nut samples contaminated with AFT and AFB1 for third period (March, April and May 2009) is given in Table 4.10. In this period, 47 unpackaged and 13 packaged pistachio nut samples were analyzed for AFT and AFB1. One (2.2 %) unpackaged pistachio nut sample was contaminated with AFT and AFB1 over the legal limit (in May) and the contamination level of AFT and AFB1 were 148.15 and 133.49 ppb, respectively. Both of AFT and AFB1 were detected in legal limits in 36.1 % (17/47) unpackaged pistachio nut samples with different contamination levels ranged from 0.007 to 3.83 ppb and from 0.007 to 3.62 ppb, respectively. The statistical results were given in Appendix E3 and Appendix E8 with respect to the unpackaged and packaged samples. There are nonsignificant difference (P>0.05) between these 3 months unpackaged pistachio nut samples with respect to the AFT and AFB1. There are nonsignificant difference (P>0.05) between these 3 months packaged pistachio nut samples, respectively.

Table 4.10. Number of unpackaged and packaged pistachio nut samples contaminated with total aflatoxin (AFT) and aflatoxin B1 (AFB1) collected in March, April and May 2009

Type of pistachio nut sample	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds	
	<10 ppb	ND	<5 ppb	ND	$< 10^4$	$> 10^4$
Unpack. (n=47)[1]	17 (36.1 %)	29 (61.7 %)	17 (36.1 %)	29 (61.7 %)	46 (97.9%)	1 (2.1%)
Packaged (n=13)	3 (23.1 %)	10 (66.9 %)	3 (23.1 %)	10 (66.9 %)	13 (100%)	-
Total (n=60)	21 (35.0 %)	39 (65 %)	21 (35.0 %)	39 (65 %)	59 (98.3%)	1 (1.7%)

[1]One sample (2.2 %, 1/47) contaminated with AFT and AFB1 over legal limit.

The number (percent) of unpackaged and packaged pistachio nut samples contaminated with AFT and AFB1 for last period (June, July and August 2008) is given in Table 4.11. In this period, 47 unpackaged and 13 packaged pistachio nut samples were analyzed for AFT and AFB1. Among 47 samples analyzed, AFT was detected in 36.2 % (17/47) unpackaged pistachio nut samples with the contamination levels ranged from 0.01 to 0.22 ppb and AFB1 was detected in 29.8 % (14/47) unpackaged pistachio nut samples with the contamination level ranged from 0.01 to 0.41 ppb. AFT and AFB1 were not detected in packaged pistachio nut samples. The statistical results were given in Appendix E4 and Appendix E9 with respect to the unpackaged and packaged samples. There are nonsignificant difference ($P>0.05$) between these 3 months unpackaged pistachio nut samples with respect to the AFT and AFB1. There are significant difference ($P<0.05$) between these 3 months packaged pistachio nut samples, respectively.

Table 4.11. Number of unpackaged and packaged pistachio nut samples contaminated with total aflatoxin (AFT) and aflatoxin B1 (AFB1) collected in June, July and August 2008

Type of pistachio nut samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Mold	
	<10 ppb	ND	<5 ppb	ND	$< 10^4$	$> 10^4$
Unpack. (n=47)	17 (36.2 %)	30 (63.8 %)	14 (29.8 %)	33 (70.2 %)	47 (100%)	-
Packaged (n=13)	-	13 (100 %)	-	13 (100 %)	13 (100%)	-
Total (n=60)	17 (28.3 %)	43 (71.7 %)	14 (23.3 %)	46 (76.7 %)	60 (100%)	-

In this study 240 pistachio nut samples were collected from June 2008 to May 2009 in different markets and groceries which were sold in Gaziantep (Table 4.12). None of the 105 (55.6 %) unpackaged and 37 (72.5 %) packaged pistachio nut samples were contaminated with aflatoxins. AFT and AFB1 were detected in 43.4 % (82/189) and 41.8 % (79/189) unpackaged pistachio nut samples, respectively, with the contamination levels ranged from 0.007 to 7.72 ppb and from 0.007 to 4.08 ppb, respectively, and only one (0.5 %) of 189 unpackaged pistachio nut sample was contained AFT and AFB1 over legal limit with contamination level 148.15 and 133.49 ppb, respectively. Both of AFT and AFB1 was detected in 27.5 % (14/51) packaged pistachio nut samples with different contamination levels ranged from 0.01 to 0.98 ppb and from0.01 to 0.79 ppb respectively. The statistical results were given in Appendix D5 and Appendix D10 with respect to the unpackaged and packaged samples. There are significant difference (P>0.05) between the four periods of unpackaged ground red pepper for AFT and AFB1. There are nonsignificant difference (P>0.05) between the four periods of unpackaged and packaged ground red pepper for AFT and AFB1.

Table 4.12. Number of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected from June 2008 to May 2009 (12 months)

Type of pistachio nut samples	Number of samples with AFT (%)		Number of samples with AFB1 (%)		Molds	
	<10 ppb	ND	<5 ppb	ND	$< 10^4$	$> 10^4$
Unpack. (n=189)[1]	82 (43.4 %)	106 (56.1 %)	79 (41.8 %)	109 (57.7 %)	187 (98.9%)	2 (1.1 %)
Packaged (n=51)	14 (27.5 %)	37 (72.5 %)	14 (27.5 %)	37 (72.5 %)	51 (100 %)	-
Total (n=240)	97 (40.4 %)	143 (59.6 %)	94 (39.2 %)	146 (60.8 %)	238 (99.2%)	2 (0.8 %)

[1]One sample (0.5 %, 1/189) contaminated with AFT and AFB1 over legal limit.

The statistical analysis of periods were performed. The results were given in Appendix D. There are nonsignificant difference (P>0.05) between the four periods of unpackaged pistachio nut in AFT. There are nonsignificant difference (P>0.05) between the four periods of unpackaged pistachio nut in AFB1. There are nonsignificant difference (P>0.05) between the four periods of packaged ground red

pepper in AFT. There are nonsignificant difference (P>0.05) between the four periods of packaged ground red pepper in AFB1.

4.1.2 Mold Count

Mold count was evaluated according to the legal limit which was 10^4 in Turkish Food Codex (Microbiological Criteria). Results were given in Table 4.8 to 4.12 for three months periods. In the first period none of the unpackaged and/or packaged pistachio nut sample was exceeding the legal limit. In the second and third period only one unpackaged sample was exceeding the legal limit while none of the packaged sample was exceeding the legal limit. In the last period none of the unpackaged and packaged sample was exceeding the legal limit. In total 0.8 % of pistachio nut sample was over the legal limit where 99.2 % sample was below the legal limit. No relation seen between the mold count and aflatoxin levels.

4.2.3 Chemical Analysis

pH analyses were carried out in packaged and unpackaged pistachio nut samples during 12 months period from June 2008 to May 2009. In the first period (September, October and November 2008) results were ranged from 3.8 to 6.21 and 48.3 % (29/60) sample values were greater than 5.0. In the second period (December 2008, and January and February 2009), results were ranged from 4.23 to 5.85 and 46.7 % (28/60) samples were greater than 5.0. In the third period (March, April and May 2009), results were ranged from 3.63 to 6.08 and 23.3 % (14/60) samples were greater than 5.0. In the last period (June, July and August 2008), results were ranged from 3.87 to 6.01 and 31.7 % (19/60) samples were greater than 5.0. The results were given in Table 4.13.

Table 4.13. Results of pH analyses in packaged and unpackaged pistachio nut samples for the four periods

pH range	1. period		2.period	
	Unpackaged (n=51)	Packaged (n=9)	Unpackaged (n=41)	Packaged (n=19)
pH < 5.0	30 (58.8 %)	3 (33.3 %)	26 (63.4 %)	14 (73.7 %)
pH >5.0	21 (41.2 %)	6 (66.6 %)	15 (36.6 %)	5 (26.3 %)

	3. period		4.period	
	Unpackaged (n=47)	Packaged (n=13)	Unpackaged (n=47)	Packaged (n=13)
pH < 5.0	34 (72.3 %)	9 (69.2 %)	31 (66 %)	10 (77 %)
pH >5.0	13 (27.7 %)	4 (30.8 %)	16 (34 %)	3 (23 %)

Water activity (a_w) analyses were also carried out in the ground red pepper samples during 12 months period from June 2008 to May 2009. The results were given in Table 4.14 for the unpackaged and packaged pistachio nuts for four periods. In the first period (September, October and November 2008) results were ranged from 0.25 to 0.78. In the second period (December 2008, and January and February 2009), results were ranged 0.37 to 0.67. In the third period (March, April and May 2009), results were ranged from 0.34 to 0.67. In the last period (June, July and August 2008), results were ranged from 0.40 to 0.62.

Table 4.14. Results of water activity (a_w) analyses in packaged and unpackaged pistachio nut samples for the four period

a_w range	1. period		2. period	
	Unpackaged (n=51)	Packaged (n=9)	Unpackaged (n=41)	Packaged (n=19)
>0.80	3 (5.9 %)	-	-	-
0.79-0.60	3 (5.9 %)	-	-	-
0.59-0.40	16 (31.4 %)	-	13 (31.7 %)	6 (31.6 %)
0.39-0.20	28 (54.9 %)	9 (100 %)	28 (68.3 %)	13 (68.4 %)
<0.19	1 (1.9 %)	-	-	-

	3. period		4. period	
	Unpackaged (n=47)	Packaged (n=13)	Unpackaged (n=47)	Packaged (n=13)
>0.80	-	-	8 (17 %)	-
0.79-0.60	-	1 (7.7 %)	-	-
0.59-0.40	6 (12.8 %)	2 (15.4 %)	4 (8.5 %)	-
0.39-0.20	39 (82.9 %)	9 (69.2 %)	32 (68.1 %)	13 (100 %)
<0.19	2 (4.3 %)	1 (7.7 %)	3 (6.4 %)	-

The commodities with the highest risk of AFT contamination include corn, peanut, cottonseed, nuts, pistachio nut, fig, spice and copra (Pittet, 1998). Natural occurrence of aflatoxins in pistachio nuts has been studied in various countries. According to the report from Mexico, 2.2 % of pistachio nut samples were contained aflatoxins higher than 20 ppb (JECFA, 1998). In Sweden, 9.5 % pistachio nut samples contained aflatoxin B1 higher than 2 ppb (Thuvander et al., 2001). According to the report of Japanese Ministry of Health, among pistachio nut samples analyzed during 1972-1989, only 2 % of samples contained aflatoxin B1 higher than 10 ppb (JECFA, 1998). According to the Food Standards Agency survey in UK, among 52 pistachio nut samples analyzed for aflatoxins using HPLC, 44 samples were not contaminated with aflatoxins (lower than limit of 2 ppb), 2

samples contained with aflatoxins between 2 and 4 ppb, 2 samples contained AFT between 4 ppb and 10 ppb, and 4 samples contained AFT higher than 10 ppb (Food Standards Agency, 2002). In the Netherlands, among 29 pistachio nut samples, AFB1 was found in 17 (58.6 %) samples ranging from 0.8 to 165 ppb (Scholten and Spanjer, 1996). In Qatar, 27.7 % analyzed pistachio nut samples were contaminated with AFT above the 20 ppb (Abdulkadar et al., 2000). Juan et al. (2008) studied on 20 pistachio nut samples for aflatoxin and found AFB1 in 45.0 % of samples was in the range of 0.04-1430 ppb. In Bahrain, 2 (66.7 %) of 3 samples were containing AFT and 1 sample (81.6 ppb) exceeded the permitted limit (allowable limit was 20 ppb in Bahrain) (Musaiger et al., 2007). According to a report from Mexico(JECFA, 1998), 2.2 % of pistachio nut samples contained with AFT higher than 20 ppb. In Iran, among 10,068 were analyzed, 3699 samples (36.7 %) were contaminated with AFB1 (Cheraghali et al., 2007). In South Korea, 1 (25.0 %) of 4 samples contained AFT which was 3.41 ppb (Chun et al., 2007).

In contrast to many crops, tree nuts for export undergo minimal or very light processing, such as blanching, and the majority of the crop are consumed as whole nuts. Any subsequent processing, such as incorporation into baked goods or conversion into marzipan is performed by the importer or ultimate consumer after aflatoxin analysis has been performed. There is thus little opportunity to reduce aflatoxin levels by artificial means and natural, consumer-acceptable methods must therefore be found. The nature of tree nut harvesting and processing, which involves considerable potential for spreading of mold spores and aflatoxins throughout the lots, mandates that the most effective method of control would be to prevent aflatoxin formation in the nuts themselves by enhancing natural resistance. The incidence of aflatoxins contamination in tree nuts is low, but their levels are quite variable and high levels may develop in a small percentage of nuts (Schatzki, 1995). The contamination of pistachio nuts with aflatoxins was high in some producer and importer countries such as the Gulf region in Bahrain, Netherlands, Qatar, Iran (Cheraghali et al., 2007; Musaiger et al., 2008). Various degrees of aflatoxin contamination with pistachio nuts have been reported where the early-split nuts were the most contaminated, late split nuts were less contaminated, and pistachio nuts with sound hulls being almost clean (Hadavi, 2005). Abdulkadar and Al-Jedah

(2002) reported that the highest incidence and levels of aflatoxin contamination was in pistachio nut without shell followed by pistachio nuts with shell.

Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA, 1998) has estimated that in a western consumer society (Europe), where food contamination levels by aflatoxins are low and the prevalence of Hepatitis B runs only at about 1 %, a tolerance level of 20 ppb for peanuts present an estimated population risk of 0.0041 cancers/year per 100,000 people. By reducing the tolerance level by half to 10 ppb, the estimated risk to the European population falls to 0.0039 cancers/year per 100,000 people. This reduction from 20 ng/g to 10 ng/g amounts to a drop in estimated risk of two additional cancers/year, per 1000,000,000 people (JECFA, 1998). The Scientific Committee for Food has noted that AFB1 is a potent genotoxic carcinogen and even at extremely low levels, contributes to the risk of liver cancer (SCF, 1996). The European Union has legislated maximum permitted levels of 4 ppb for AFT (B_1, B_2, G_1 and G_2) and 2 ppb for AFB1 in various nuts for direct human consumption, including pistachio nuts (EC, 2006a). In recent years an increasing number of notifications through the world for food and those maximum levels have been regularly exceeded in pistachio nuts from some third countries (RASFF, 2006). Such contamination constituents a threat to public health, and special conditions governing pistachio nuts imported from certain third countries due to contamination risks of these products by aflatoxins, are laid down in Commission Decision 2006/504/EC (EC, 2006b). In this study, 97 and 94 of 240 pistachio nut samples contaminated with AFT and AFB1, respectively, with the contamination level ranging from 0.007 to 148.15 ppb and from 0.007 to 133.49 ppb respectively. Therefore only 1.7 % (4/240) and 4.2 % (10/240) of unpackaged pistachio nut samples were exceeding EU legal limits (4 and 2 ppb) while none of the packaged pistachio nut samples were exceeding the legal limits.

Aflatoxin is stable to dry heat up to its melting point of 250^{o}C. However some degradation of aflatoxins can occur at extended exposure to temperatures lower than 250^{o}C, with the percent reduction being dependent on time and temperature (Bullerman and Bianchini, 2007). Pluyer et al. (1987) reported that 30-40 % of aflatoxin in peanuts was destroyed when oven-roasted at 150^{o}C for 30 min. Roasting naturally contaminated whole pistachio nut kernels in-shell at 150^{o}C for 30 min at

laboratory scale resulted in 37-81 % loss of aflatoxins (Yazdanpanah et al., 2005). Naturally occurring aflatoxins were more resistant to heat degradation than those artificially spiked to pistachio nuts (Afrino et al., 2009). Afrino et al. (2009) analyzed the aflatoxins level in roasted pistachio nuts that were exported from other countries. They reported that 19-50 % of roasted pistachio nuts analyzed contained AFB1 at low levels between 0.12 and 0.29 ppb. All positive samples originated from Iran, while pistachio nuts from USA, Turkey and Spain tested negative for aflatoxins. None of the samples exceeded the maximum tolerable limit for AFB1 set at 2 ppb by current European Union regulations. Analysis of 523 pistachio nut samples in Turkey revealed the mean of AFB1 and the maximum level detected was 113 ppb (Ministry of Agriculture and Rural Affairs, Republic of Turkey, 2002).

The importance of RH and temperature on aflatoxin production in nuts and establishes limits for both these parameters. A RH of 97 % accompanied by temperatures in the range of 25-30^{o}C was shown to promote aflatoxin production in infected nuts. Unfortunately conditions are representative of the harvesting season. Reduction of RH and/or temperature may not be an economic option. Air and or mechanical drying of the nuts prior to storage could be used and would limit mold growth and toxin formation. In this respect whole shelled and in-shell nuts should be dried to a moisture content of approximately 4.5 % and 5.0 %, respectively (Arrus et al., 2005).

Since pistachio nuts are among the commodities with the highest risk of aflatoxins contamination (Pittet, 1998), a prevention program should be established considering various steps from cultivation through harvesting, post-harvesting, processing, storage, transporting and marketing. The contamination is due to the fact that may be some food safety and quality standards; good agricultural practices (GAPs), good manufacturing practices (GMPs) and the hazard analysis critical control point (HACCP) system are not applied and performed in ground red pepper but in some cases pistachio nuts to control growth of molds and mycotoxin production during harvesting, processing, distribution and storage periods. The higher the contamination level of aflatoxin, in ground red pepper samples was quite high and the contaminated ground red pepper samples was 30.0 % with contamination level ranging from 0.087 to 97.4 ppb. There is also possibility of

aflatoxin production in/on pistachio nuts from harvesting to consumption. Since 40.4 % (97/240) pistachio nut samples was contaminated with aflatoxin with the contamination level ranging from 0.007 to 148.15 ppb.

This situation should spur Turkish authorities to devise prevention measures and set programs for surveillance of mycotoxins in ground red pepper and pistachio nuts. Research on the prevention of mold contamination and growth, and mycotoxin formation mostly in ground red pepper from harvesting to consumption are needed. In recent years, health and agriculture authorities have implemented strict regulations in order to control aflatoxin contamination. Efforts have been made to manage aflatoxins contamination by promoting Good Agricultural Practice (GAP) principles in productions and Hazard Analysis and Critical Control Point (HACCP) principles from harvesting to consumption. When preventive action cannot be achieved, corrective action needs to be done. Removal of aflatoxin contaminated nuts by means of physical segregation is not the most effective control measure for reducing levels of aflatoxin in a lot to an acceptable level.

CHAPTER 5

CONCLUSION

The data obtained from this study revealed that 21.7 % and 32.5 % of the ground red pepper samples exceed the legal limit of AFT (10 ppb) and AFB1 (5 ppb), respectively, indicated in Turkish Food Codex (Regulation No:2008/26) and EU (Commission Regulation, EC No. 1881/2006 and EC No. 1126/2007). Aflatoxins are common contamination of ground red pepper and can be considered to be a main concern for public health. Therefore public health authorities should urgently pay attention to aflatoxin particularly by monitoring ground red pepper and ground red pepper products from harvesting to consumption and informing ground red pepper producers. Ground red pepper samples examined have great risk on public health and economics. Some measurements are necessary to produce high quality ground red pepper. 1.7% and 4.2% of pistachio nut sample exceed the EU legal limit of AFT (4 ppb) and AFB1 (2 ppb). Pistachio nut samples examined did not have any risk on public health and economics. But there was a possibility of mold contamination and aflatoxin production from harvesting to consumption. Therefore pistachio nuts production also needs attention to prevent mold contamination and growth, and mycotoxin production. These measures can be avoiding contact with soil during harvesting and drying, and prevent contamination of molds during harvesting, processing and storage periods. In addition, packaging is also a good measure to supply attractive and healthy products in hygienic condition. This study provides useful information about the risk of mycotoxin hazard and hopes to rise the consciousness among consumers, researchers, farmers and traders about the importance of improve processing methods (harvesting, drying, packaging, transportation and storage) and to establish a monitoring program on food and the necessity to obtain more data on the distribution and contamination levels of aflatoxins in human food, like ground red peppers and pistachio nuts. The data obtained from this monitoring can be used as a basis for risk analysis of aflatoxin, thereby maintaining the aflatoxin at the lowest possible levels.

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APPENDICES A

Results of Aflatoxin Comtamination of Ground Red Pepper

Table A.1 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in September 2008

	AFT	AFB1
	Unpackaged	
1	0,42	0,37
2	1,16	1,10
3	2,39	2,22
4	18,48	17,75
5	10,14	9,76
6	1,59	1,53
7	1,89	1,14
8	35,70	34,90
9	57,30	55,90
10	8,35	8,18
11	2,41	2,37
12	1,28	1,22
13	0,34	0,31
14	4,62	4,24
	Packaged	
1	1,80	1,75
2	1,75	1,64
3	0,91	0,89
4	2,06	1,95
5	0,56	0,50
6	1,20	1,15

Table A.2 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in October 2008

	AFT	AFB1
	Unpackaged	
1	2,05	1,83
2	1,78	1,64
3	18,77	17,97
4	1,54	1,49
5	0,26	0,08
6	11,03	10,60
7	16,97	15,51
8	2,08	2,02
9	0,81	0,76
10	25,62	21,55
11	0,50	0,30
	Packaged	
1	0,47	0,43
2	0,68	0,66
3	0,08	0,08
4	1,04	0,96
5	0,45	0,44
6	0,47	0,47
7	0,66	0,63
8	0,67	0,63
9	0,73	0,69

Table A.3 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in November 2008

	AFT	AFB1
	Unpackaged	
1	0,65	0,60
2	2,03	1,69
3	4,43	3,81
4	1,58	1,41
5	0,133	0,066
6	1,65	1,61
7	1,09	1,03
8	1,19	1,12
9	0,36	0,35
10	0,95	0,89
11	0,63	0,56
12	0,56	0,41
	Packaged	
1	1,03	0,96
2	0,91	0,86
3	0,73	0,68
4	1,41	1,36
5	0,124	0,096
6	0,127	0,094
7	0,44	0,42
8	0,24	0,22

Table A.4 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in December 2008

	AFT	AFB1
	Unpackaged	
1	0,96	0,89
2	0,69	0,61
3	7,98	7,52
4	8,27	7,63
5	1,39	1,32
6	0,79	0,75
7	5,49	4,89
8	6,61	5,95
9	8,20	6,99
10	10,38	6,84
11	3,99	3,28
12	1,19	0,90
13	4,38	4,15
	Packaged	
1	0,126	0,126
2	0,138	0,138
3	0,52	0,37
4	0,25	0,22
5	0,09	0,09
6	0,09	0,07
7	0,69	0,58

Table A.5 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in January 2009

	AFT	AFB1
	Unpackaged	
1	0,26	0,24
2	0,11	0,085
3	2,42	2,33
4	2,01	1,81
5	1,71	1,61
6	19,96	18,82
7	1,73	1,6
8	0,25	0,23
9	0,56	0,51
10	0,99	0,88
11	0,087	0,067
12	4,26	3,94
13	2,88	1,14
14	2,05	1,83
15	2,26	2,01
16	4,02	3,57
17	3,18	3,05
18	15,86	12,14
	Packaged	
1	9,32	6,86
2	0,21	0,14

Table A.6 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in February 2009

	AFT	AFB1
	Unpackaged	
1	0,10	0,08
2	0,11	0,11
3	0,35	0,33
4	1,25	1,19
5	1,38	1,30
6	1,46	0,55
7	2,01	1,89
8	2,48	2,35
9	2,65	2,52
10	2,74	1,66
11	6,00	5,65
12	9,49	9,00
13	10,80	7,48
14	12,12	6,76
	Packaged	
1	0,36	0,32
2	0,38	0,37
3	0,39	0,34
4	0,99	0,37
5	1,15	1,00
6	1,16	0,43

Table A.7 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in March 2009

	AFT	AFB1
	Unpackaged	
1	0,51	0,45
2	2,03	1,96
3	2,50	2,41
4	3,07	2,89
5	3,69	3,48
6	3,74	3,60
7	4,98	4,80
8	5,71	5,45
9	7,69	7,26
10	7,94	7,38
11	11,81	10,90
12	13,05	12,14
13	13,07	12,44
14	15,35	14,30
15	20,03	18,71
	Packaged	
1	0,06	0,06
2	0,53	0,50
3	0,82	0,33
4	1,95	1,58
5	8,90	8,25

Table A.8 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in April 2009

	AFT	AFB1
	Unpackaged	
1	1,31	1,25
2	1,75	1,66
3	2,45	2,25
4	5,89	5,69
5	6,15	5,85
6	6,61	6,16
7	6,71	6,39
8	6,87	5,02
9	8,42	8,25
10	9,93	4,08
11	10,09	9,45
12	12,91	11,74
13	17,86	15,37
	Packaged	
1	0,19	0,12
2	0,45	0,42
3	0,59	0,53
4	0,99	0,91
5	1,04	0,96
6	2,21	1,85
7	4,76	1,98

Table A.9 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in May 2009

	AFT	AFB1
	Unpackaged	
1	0,35	0,25
2	0,94	0,89
3	1,10	1,07
4	1,23	1,18
5	1,30	1,26
6	1,58	1,51
7	1,79	1,64
8	2,88	2,76
9	3,31	2,80
10	4,41	4,17
11	6,38	6,00
12	17,28	16,23
13	22,95	21,39
14	30,20	28,24
15	36,49	33,49
	Packaged	
1	1,18	1,06
2	1,85	1,75
3	2,59	2,45
4	2,65	2,54
5	2,73	2,67

Table A.10 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in June 2009

	AFT	AFB1
	Unpackaged	
1	0,52	0,52
2	0,72	0,72
3	2,23	2,10
4	2,62	2,50
5	3,05	2,95
6	3,11	3,00
7	8,54	7,98
8	11,56	10,90
9	14,04	13,11
10	15,73	14,49
11	37,03	33,17
12	72,26	67,16
13	97,40	89,99
	Packaged	
1	1,24	0,79
2	1,26	0,81
3	1,56	1,49
4	1,66	1,59
5	2,01	2,01
6	8,37	8,02
7	17,54	15,46

Table A.11 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in July 2008

	AFT	AFB1
	Unpackaged	
1	2,04	1,98
2	5,53	5,32
3	7,64	6,52
4	10,72	9,65
5	12,95	12,10
6	13,95	12,89
7	14,61	13,52
8	14,74	13,72
9	15,44	14,23
10	15,62	14,45
11	25,12	23,94
12	29,18	5,32
13	34,70	6,31
14	44,24	41,56
15	54,94	51,23
16	69,84	64,72
	Packaged	
1	0,15	0,15
2	0,21	0,21
3	0,92	0,80
4	1,60	1,10

Table A.12 Level of unpackaged and packaged ground red pepper samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in August 2008

	AFT	AFB1
	Unpackaged	
1	4,03	3,80
2	47,83	45,38
3	55,65	51,56
4	1,61	1,37
5	13,66	12,32
6	9,59	6,51
7	64,33	61,16
8	32,94	28,79
9	65,80	62,42
10	5,32	5,23
11	6,40	6,29
12	6,89	6,56
13	3,58	3,36
14	4,34	3,18
15	5,93	4,31
16	6,83	6,71
	Packaged	
1	0,38	0,28
2	0,71	0,70
3	2,04	1,92
4	1,28	1,10

APPENDICES B

Results of Aflatoxin Comtamination of Pistachio Nut

Table B.1 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in September 2008

	AFT	AFB1
	Unpackaged	
1	_	_
2	0,02	0,02
3	0,93	0,56
4	0,03	0,025
5	0,08	0,04
6	_	_
7	_	_
8	0,026	0,026
9	0,03	0,03
10	0,023	0,023
11	0,01	0,01
12	0,013	0,013
13	0,023	0,023
14	_	_
15	0,24	0,22
16	0,10	0,09
	Packaged	
1	0,0125	0,0125
2	_	_
3	_	_
4	0,054	0,054

Table B.2 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in October 2008

	AFT	AFB1
	Unpackaged	
1	3,69	3,31
2	0,04	0,04
3	3,90	3,69
4	4,05	3,86
5	0,014	0,014
6	0,025	0,025
7	0,028	0,028
8	0,024	0,024
9	0,38	0,32
10	0,03	0,03
11	0,47	0,44
12	1,85	1,80
13	0,04	0,04
14	_	_
15	2,95	2,81
16	1,93	1,63
17	1,33	1,16
18	6,44	1,68
19	2,80	2,67
20	2,75	2,63

Table B.3 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in November 2008

	AFT	AFB1
	Unpackaged	
1	0,22	0,20
2	_	_
3	_	_
4	_	_
5	0,025	0,020
6	_	_
7	_	_
8	0,015	0,015
9	0,013	0,013
10	_	_
11	0,023	0,019
12	_	_
13	_	_
14	0,017	0,016
15	0,050	0,030
	Packaged	
1	0,050	0,030
2	0,040	0,030
3	0,070	0,030
4	0,020	0,020
5	0,044	0,027

Table B.4 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in December 2008

	AFT	AFB1
	Unpackaged	
1	0,045	0,040
2	0,23	0,20
3	_	_
4	_	_
5	0,085	0,080
6	_	_
7	_	_
8	_	_
9	1,91	1,63
10	_	_
11	_	_
12	_	_
13	_	_
14	_	_
	Packaged	
1	0,160	0,130
2	0,05	0,05
3	0,076	0,06
4	_	_
5	_	_
6	0,41	0,36

Table B.5 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in January 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	0,05	0,05
3	0,74	0,66
4	7,72	4,08
5	_	_
6	_	_
7	_	_
8	_	_
9	_	_
10	_	_
11	_	_
12	_	_
13	_	_
14	_	_
	Packaged	
1	_	_
2	_	_
3	0,019	0,019
4	_	_
5	_	_
6	_	_

Table B.6 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in February 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	_	_
3	_	_
4	_	_
5	_	_
6	_	_
7	2,78	2,57
8	0,15	0,12
9	_	_
10	_	_
11	_	_
12	_	_
13	_	_
	Packaged	
1	_	_
2	_	_
3	_	_
4	_	_
5	_	_
6	_	_
7	_	_

Table B.7 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in March 2009

	AFT	AFB1
	Unpackaged	
1	–	–
2	–	–
3	–	–
4	0,043	0,013
5	–	–
6	0,10	0,06
7	0,045	0,04
8	–	–
9	–	–
10	0,01	0,01
11	0,007	0,007
12	0,014	0,014
13	0,026	0,026
	Packaged	
1	0,069	0,016
2	–	–
3	–	–
4	0,98	0,79
5	–	–
6	–	–
7	–	–

Table B.8 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in April 2009

	AFT	AFB1
	Unpackaged	
1	0,11	0,03
2	3,83	3,62
3	–	–
4	–	–
5	–	–
6	0,045	0,045
7	–	–
8	–	–
9	–	–
10	0,018	0,018
11	–	–
12	0,014	0,010
13	–	–
14	0,034	0,018
15	0,054	0,030
16	0,280	0,100
	Packaged	
1	–	–
2	–	–
3	–	–
4	0,033	0,033

Table B.9 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in May 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	_	_
3	_	_
4	_	_
5	_	_
6	_	_
7	_	_
8	_	_
9	_	_
10	148,15	133,49
11	_	_
12	_	_
13	_	_
14	_	_
15	0,35	0,20
16	0,39	0,24
17	_	_
18	_	_
	Packaged	
1	_	_
2	_	_

Table B.10 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in June 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	_	_
3	0,06	0,06
4	_	_
5	0,03	0,03
6	_	_
7	0,15	0,15
8	_	_
9	_	_
10	_	_
11	_	_
12	_	_
13	_	_
14	_	_
15	_	_
16	0,04	0,04
	Packaged	
1	_	_
2	_	_
3	_	_
4	_	_

Table B.11 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in July 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	0,12	0,12
3	_	_
4	_	_
5	_	_
6	_	_
7	_	_
8	_	_
9	_	_
10	_	_
11	_	_
12	_	_
13	_	_
14	_	_
	Packaged	
1	_	_
2	_	_
3	_	_
4	_	_
5	_	_
6	_	_

Table B.12 Level of unpackaged and packaged pistachio nut samples contaminated with total aflatoxins (AFT) and aflatoxin B1 (AFB1) collected in August 2009

	AFT	AFB1
	Unpackaged	
1	_	_
2	_	_
3	0,01	0,01
4	0,41	0,22
5	0,04	
6	0,01	0,01
7	0,03	0,03
8	0,04	_
9	_	_
10	_	_
11	0,02	0,02
12	_	_
13	0,06	0,06
14	0,02	0,02
15	0,096	0,07
16	_	_
17	0,02	0,02
	Packaged	
1	_	_
2	_	_
3	_	_

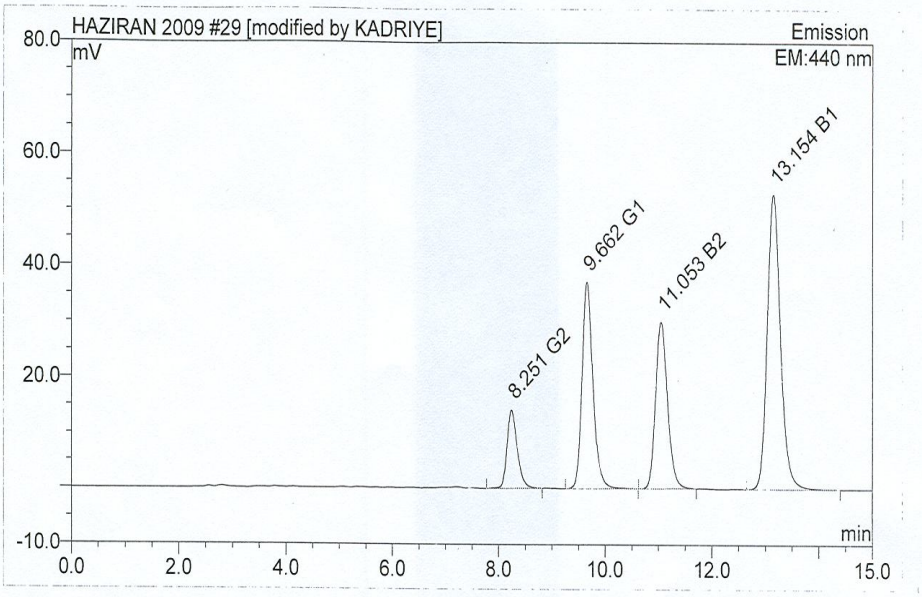
APPENDICES C

Figure of a Chromatogram

GAZIANTEP IL KONTROL LABORATUVAR MÜDÜRLÜGÜ

MIKOTOKSIN ANALIZ LABORATUVARI

Sample Name:	STD 01	**Sample No.:**	29
Sequence Name:	HAZIRAN 2009		
Program Method:	AFLATOKSIN	**Injection vol.:**	100.0
Quantitation Method:	AFLA 09	**Dilution Factor:**	1.0000
Date Time Collected:	1.6.2009 9:12	**Sample Wt.:**	1.0000
System Operator:	Ebru SET	**Sample Amt.:**	1.0000

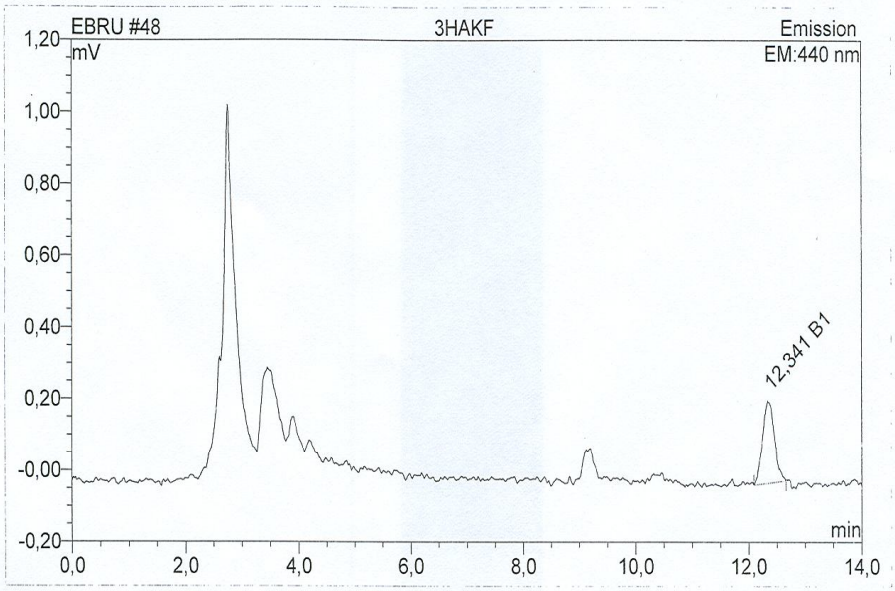


Peak No.	Component Name	Ret.Time min	Area mV*min	Height mV	Amount	Relative Amount %
1	G2	8.000	3.171	14.186	1.4033	11.24
2	G1	9.000	9.010	37.130	4.6391	37.14
3	B2	10.400	7.690	29.999	1.4499	11.61
4	B1	12.979	15.288	52.894	4.9970	40.01
	TOPLAM				**12.4893**	

GAZIANTEP IL KONTROL LABORATUVAR MÜDÜRLÜGÜ

MIKOTOKSIN ANALIZ LABORATUVARI

Sample Name:	3HAKF	**Sample No.:**	48
Sequence Name:	EBRU		
Program Method:	AFLATOKSIN	**Injection vol.:**	100,0
Quantitation Method:	AFLA 12	**Dilution Factor:**	2,0000
Date Time Collected:	26.6.2008 15:31	**Sample Wt.:**	1,0000
System Operator:	Ebru SET	**Sample Amt.:**	1,0000

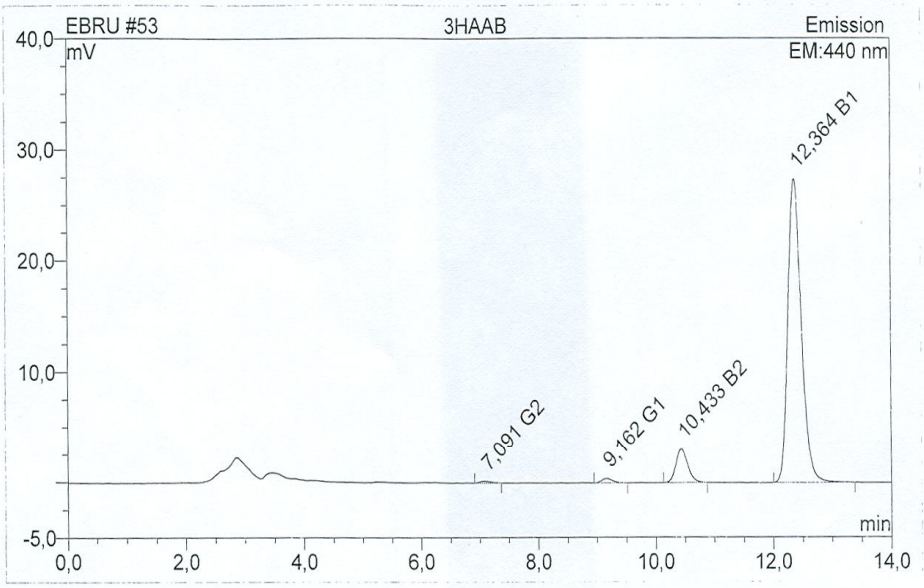


Peak No.	Component Name	Ret.Time min	Area mV*min	Height mV	Amount	Relative Amount %
1	B1	12,500	0,057	0,231	**0,0568**	100,00
n.a.	G2	7,655	n.a.	n.a.	**n.a.**	n.a.
n.a.	G1	8,910	n.a.	n.a.	**n.a.**	n.a.
n.a.	B2	10,500	n.a.	n.a.	**n.a.**	n.a.
	TOPLAM				**0,0568**	

GAZIANTEP IL KONTROL LABORATUVAR MÜDÜRLÜGÜ

MIKOTOKSIN ANALIZ LABORATUVARI

Sample Name:	3HAAB	**Sample No.:**	53
Sequence Name:	EBRU		
Program Method:	AFLATOKSIN	**Injection vol.:**	100,0
Quantitation Method:	AFLA 12	**Dilution Factor:**	4,0000
Date Time Collected:	26.6.2008 16:45	**Sample Wt.:**	1,0000
System Operator:	Ebru SET	**Sample Amt.:**	1,0000



Peak No.	Component Name	Ret.Time min	Area mV*min	Height mV	Amount	Relative Amount %
1	G2	7,655	0,040	0,174	**0,0991**	0,63
2	G1	8,910	0,090	0,420	**0,2545**	1,62
3	B2	10,500	0,712	3,056	**0,8887**	5,65
4	B1	12,500	7,218	27,398	**14,4912**	92,10
	TOPLAM				**15,7336**	

APPENDICES D

Results of Statistical Analysis of Ground Red Pepper

Table D.1 One-way ANOVA of unpackaged ground red pepper for AFT and AFB1 for 1.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	553,5139	2	276,7569	2,121566	0,135436	3,275898
Within groups	4435,279	34	130,4494			
Total	4988,793	36				
AFB1						
Among groups	521,1215	2	260,5607	2,158215	0,131098	3,275898
Within groups	4104,811	34	120,7297			
Total	4625,932	36				

Table D.2 One-way ANOVA of unpackaged ground red pepper for AFT and AFB1 for 2.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	8,936264	2	4,468132	0,218528	0,804609	3,219942
Within groups	858,7514	42	20,44646			
Total	867,6876	44				
AFB1						
Among groups	8,7086	2	4,3543	0,303436	0,739882	3,219942
Within groups	602,6983	42	14,34996			
Total	611,4069	44				

Table D.3 One-way ANOVA of unpackaged ground red pepper for AFT and AFB1 for 3.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	15,2827	2	7,641351	0,112872	0,893549	3,231727
Within groups	2707,975	40	67,69938			
Total	2723,258	42				
AFB1						
Among groups	22,65277	2	11,32639	0,196393	0,822477	3,231727
Within groups	2306,887	40	57,67218			
Total	2329,54	42				

Table D.4 One-way ANOVA of unpackaged ground red pepper for AFT and AFB1 for 4.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	59,42128	2	29,71064	0,049765	0,951509	3,219942
Within groups	25074,93	42	597,0222			
Total	25134,35	44				
AFB1						
Among groups	4,3991	2	2,19955	0,004183	0,995826	3,219942
Within groups	22086,27	42	525,8637			
Total	22090,67	44				

Table D.5 One-way ANOVA of unpackaged ground red pepper for AFT and AFB1 between four periods

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	8365,397	3	2788,466	13,72973	4,83E-08	2,659052
Within groups	33714,09	166	203,0969			
Total	42079,49	169				
AFB1						
Among groups	6380,671	3	2126,89	11,90468	4,22E-07	2,659052
Within groups	29657,55	166	178,66			
Total	36038,22	169				

Table D.6 One-way ANOVA of packaged ground red pepper for AFT and AFB1 for 1.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	2,681201	2	1,3406	7,046631	0,004826	3,492828
Within groups	3,80494	20	0,190247			
Total	6,486141	22				
AFB1						
Among groups	2,458579	2	1,229289	7,018705	0,004906	3,492828
Within groups	3,502895	20	0,175145			
Total	5,961474	22				

Table D.7 One-way ANOVA of packaged ground red pepper for AFT and AFB1 for 2.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	32,42139	2	16,21069	4,56192	0,033609	3,885294
Within groups	42,64177	12	3,55348			
Total	75,06315	14				
AFB1						
Among groups	17,49727	2	8,748637	4,539298	0,034044	3,885294
Within groups	23,12773	12	1,927311			
Total	40,625	14				

Table D.8 One-way ANOVA of packaged ground red pepper for AFT and AFB1 for 3.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	3,236634	2	1,618317	0,319348	0,731778	3,738892
Within groups	70,94597	14	5,067569			
Total	74,1826	16				
AFB1						
Among groups	5,469441	2	2,73472	0,725124	0,501589	3,738892
Within groups	52,79938	14	3,771384			
Total	58,26882	16				

Table D.9 One-way ANOVA of packaged ground red pepper for AFT and AFB1 for 4.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	56,91553	2	28,45776	1,475868	0,267262	3,885294
Within groups	231,3846	12	19,28205			
Total	288,3002	14				
AFB1						
Among groups	46,83327	2	23,41664	1,515049	0,25901	3,885294
Within groups	185,4723	12	15,45603			
Total	232,3056	14				

Table D. 10 One-way ANOVA of packaged ground red pepper for AFT and AFB1 between four periods

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	40,19985	3	13,39995	1,991741	0,123739	2,743711
Within groups	444,0321	66	6,727759			
Total	484,2319	69				
AFB1						
Among groups	32,01659	3	10,6722	2,089106	0,110060	2,743711
Within groups	337,1609	66	5,108498			
Total	369,1775	69				

APPENDICES E

Results of Statistical Analysis of Pistachio Nut

Table E.1 One-way ANOVA of unpackaged pistachio nut for AFT and AFB1 for 1.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	30,2397	2	15,11985	10,77774	0,000136	3,190727
Within groups	67,33813	48	1,402878			
Toplam	97,57783	50				
AFB1						
Among groups	19,47407	2	9,737037	12,3662	4,66E-05	3,190727
Within groups	37,79477	48	0,787391			
Toplam	57,26884	50				

Table E.2 One-way ANOVA of unpackaged pistachio nut for AFT and AFB1 for 2.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	1,616815	2	0,808408	0,469657	0,628797	3,244818
Within groups	65,40834	38	1,721272			
Toplam	67,02516	40				
AFB1						
Among groups	0,298195	2	0,149097	0,236646	0,790425	3,244818
Within groups	23,94161	38	0,630042			
Toplam	24,2398	40				

Table E.3 One-way ANOVA of unpackaged pistachio nut for AFT and AFB1 for 3.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	731,3197	2	365,6599	0,776097	0,466396	3,209278
Within groups	20730,7	44	471,1523			
Toplam	21462,02	46				
AFB1						
Among groups	592,4312	2	296,2156	0,774174	0,467263	3,209278
Within groups	16835,35	44	382,6216			
Toplam	17427,78	46				

Table E.4 One-way ANOVA of unpackaged pistachio nut for AFT and AFB1 for 4.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0,011116	2	0,005558	1,287973	0,286024	3,209278
Within groups	0,189868	44	0,004315			
Toplam	0,200983	46				
AFB1						
Among groups	0,003075	2	0,001538	0,796171	0,457589	3,21448
Within groups	0,083046	43	0,001931			
Toplam	0,086122	45				

Table E.5 One-way ANOVA of unpackaged pistachio nut for AFT and AFB1 between four periods

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	307,9946	3	102,6649	0,863974	0,460886	2,654237
Within groups	21626,83	182	118,8287			
Toplam	21934,82	185				
AFB1						
Among groups	254,9741	3	84,99137	0,878583	0,453307	2,654513
Within groups	17509,38	181	96,73689			
Toplam	17764,35	184				

Table E.6 One-way ANOVA of packaged pistachio nut for AFT and AFB1 for 1.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0,001764	2	0,000882	1,619656	0,273864	5,143253
Within groups	0,003267	6	0,000545			
Toplam	0,005032	8				
AFB1						
Among groups	0,000258	2	0,000129	0,379063	0,699801	5,143253
Within groups	0,002042	6	0,00034			
Toplam	0,0023	8				

Table E.7 One-way ANOVA of packaged pistachio nut for AFT and AFB1 for 2.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0,05389	2	0,026945	3,547094	0,053081	3,633723
Within groups	0,121541	16	0,007596			
Toplam	0,17543	18				
AFB1						
Among groups	0,039894	2	0,019947	3,435388	0,057374	3,633723
Within groups	0,092901	16	0,005806			
Toplam	0,132795	18				

Table E.8 One-way ANOVA of packaged pistachio nut for AFT and AFB1 for 3.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0,067417	2	0,033708	0,416781	0,670106	4,102821
Within groups	0,808778	10	0,080878			
Toplam	0,876194	12				
AFB1						
Among groups	0,03893	2	0,019465	0,365627	0,702664	4,102821
Within groups	0,532368	10	0,053237			
Toplam	0,571297	12				

Table E.9 One-way ANOVA of packaged pistachio nut for AFT and AFB1 for 4.period

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0	2	0	65535	#SAYI!	4,102821
Within groups	0	10	0			
Toplam	0	12				
AFB1						
Among groups	0	2	0	65535	#SAYI!	4,102821
Within groups	0	10	0			
Toplam	0	12				

Table E.10 One-way ANOVA of packaged pistachio nut for AFT and AFB1 between four periods

ANOVA						
Variance analysis	*SS*	*df*	*MS*	*F*	*P-değeri*	*F ölçütü*
Among groups	0,045642	3	0,015214	0,719907	0,54481	2,790008
Within groups	1,056656	50	0,021133			
Toplam	1,102298	53				
AFB1						
Among groups	0,027794	3	0,009265	0,655765	0,583132	2,790008
Within groups	0,706392	50	0,014128			
Toplam	0,734185	53				