T.C.
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THE AMOUNT OF FUMONISINS IN PEANUTS FROM A FRAME OF OPEN SALE IN ISTANBUL

MASTER THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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TEZ YAZIM KILAVUZU UYGUNLUK ONAYI

"Açıkta Satılan Fıstık Örneklerinin Fumonisin miktarlar" adlı Yüksek Lisans tezi, Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Lisansüstü Tez Yazım Kılavuzuna uygun olarak hazırlanmıştır.

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ABBREVIATIONS

FB1	Fumonisin B1
FB2	Fumonisin B2
PPE	Porcine Pulmonary Edema
LEM	Leukoencephalomalacia
IARC	International Agency for Research on Cancer
MOE	Ministry of Education
SPP.	Species Plural
FAO	The Food and Agricultural Organization
ELISA	Enzyme-Linked ImmunoSorbent Assay
TFC	Turkish Food Codex
HPLC	High Performance Liquid Chromatography
TLC	Total Layer Chromatography
ZEN	Zearalenon
DAS	Diacetoxyscripenol
DON	Deoxynivalenol
NIV	Nivalenol
OTA	Ochratoxin A
AF	Aflatoxin

ABSTRACT

Salman T. (2018).The Amount Of Fumonisins in Peanuts from A Frame of Open Sale in İstanbul, Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, Master Thesis. Istanbul

Food safety is one of the most important topics for human health and sanitation.It is influenced by many factors throughout the processes of production, packaging, storing and delivery to the end-user. One of these factors is mycotoxins which are caused by fungi. In order to ensure the safety of food and the production of healthy and reliable foods, the necessary rules must be followed and measures should be taken during the production, transportation, storage, distribution and consumption stages of food. In particular, compliance with HACCP principles is essential for delivering reliable food to end users. The HACCP system ensures quality and safe food production at international standards in every process from production to consumption.The main objective of this study is to indicate the amount of Fumonisins mycotoxins in the peanuts that are open-sold. For the detection of fumonisin, Elisa Kit is used. The results of the study are analyzed. In our study, 45 pieces of peanut samples sold in the province of Istanbul were collected and the amount of fumonisin was investigated by using the Elisa kit. According to our study, it was determined that the amount of fumonisin in all samples collected was in accordance with the limit determined by Turkish Food Codex.

Keywords: Fumonisin, Mycotoxin, Food Safety, Peanut

ABSTRACT (TURKISH)

Salman T. (2018). İstanbul İlinde Açıkta Satılan Fıstıkların Fumonisin Miktarlarının değerlendirilmesi,

Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik ABD, Yüksek Lisans Tezi, İstanbul

Global anlamda besin güvenliği insan sağlığı için en önemli konulardan birini oluşturmaktadır.Besin güvenliği üretim,hazırlama ,depolama ve son kullanıcıya ulaşana kadar bir çok etmenden etkilenmektedir.Bu faktörlerden biri mantarların neden olduğu mikotoksinlerdir. Gıda güvenliğini ve sağlıklı ve güvenilir gıdaların üretimini sağlamak için gerekli kurallara uyulmalı ve gıdaların üretimi, nakliyesi, depolanması, dağıtımı ve tüketim aşamaları sırasında önlemler alınmalıdır. Özellikle, HACCP ilkelerine uygunluk, son kullanıcılara güvenilir yiyecekler sunmak için esastır. HACCP sistemi, üretimden tüketime kadar her süreçte uluslararası standartlarda kaliteli ve güvenli gıda üretimi sağlamaktadır. Bu çalışmanın temel amacı, açık fıstıklarda yer alan Fumonisins mikotoksin miktarını göstermektir. Fumonisin tespiti için Elisa Kit kullanılır. Çalışmanın sonuçları analiz edildi. Çalışmamızda, İstanbul ilinde satılan 45 adet yerfıstığı örneği toplanmış ve Elisa kiti kullanılarak fumonisin miktarı araştırılmıştır. Çalışmamıza göre, toplanan tüm numunelerdeki fumonisin miktarının, Türk Gıda Kodeksi tarafından belirlenen limite uygun olduğu tespit edildi.

Anahtar Kelimeler: Fumonisin, Mikotoksin, Gıda güvenliği, Yer fıstığı

1.INTRODUCTION

Food safety and sanitation is as important for human health as ever. Thanks to many technological developments and new methods, an important progress is made on food safety. Yet, especially open-sale consumer products pose serious threats for human health.Microbial spoilage on the nutrition causes serious health problems. Therefore, it is important to maintain food safety for protection of human health and persistence of life quality.

2.GENERAL INFORMATION

Molds that can spread on nutrition and metabolites caused by them can cause health problems. Literature on molds and their effects is very-well contributed in Turkey as well as globally. Yet, the effect of molds is not only apparent on human health, as they can also cause economic losses.

Molds can reproduce and spread on nutrition in proper conditions (in terms of temperature and humidity). Thus, nutrition exposes to both physical and chemical alterations. These molds can produce extremely toxic metabolites. These metabolites which potentially causes serious health problems are called mycotoxins (1).

2.1.MYCOTOXIN

Mycotoxins are fungal metabolites that are produced in certain temperature and humidity conditions by certain fungi types such as *Aspergillus, Penicillium, Fusarium, Alternaria*. The most-commonly encountered mycotoxins are listed as follows; Aflatoxins, Ochratoxins, Trichothecene, Zearalenone, Patulin and Fumonisins (2).

Each mycotoxin can affect different biological structures (Table 1). As each mycotoxin can reproduce on a different living organism, the secondary metabolites produced by them can also have different effects on different organisms.

Table 1. Fungus and infection infected mediums.

Plant		Stored product	
Claviceps purpurea	*Helminthosporium biseptatum*	*Aspergillus flavus*	*P.urticae*
Sclerotinia sclerotiorum		*A.parasiticus*	*P.verruculosum*
Fusarium graminearum		*A.ochraceus*	*P.puberulum*
(Gibberella zeae)		*A.clavatus*	*P.expansum*
Rhizoctonia leguminicola		*A.fumigatus*	*P.rugulosum*
Aspergillus flavus		*A.rubrum*	*P.palitans*
Rotting organic matter		A.chevalieri	P.roqueforti
Pithomyces chartarum	*Fusarium graminearum*	*Penicillium islandicum*	*P. purpurogenum*
Stachybotrys atra	*Chaetomium globosum*	*P.citrinum*	*Chaetomium globosum*
Periconia minutissima	*Dendrodochium toxicum*	*P.rubrum*	*Fusarium graminearum*
Fusarium sporotrichoides	*Myrothecium verrucaria*	*P.citreoviride*	*F.tricinctum*
Cladosporium spp.	*Trichothecium roseum*	*P.cyclopium*	*F.nivale*
Alternaria longipes	*Trichoderma viride*	*P.viridicatum*	*F.moniliforme*

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The word mycotoxin is originated from the Greek words mykes (Greek, fungi) and toxicum (Latin, toxic). Mycotoxins are known as secondary metabolites. There are 400 known mold types which produce mycotoxins in optimum temperature and humidity conditions. Among these, the species that belongs to Aspergillus, Penicillium and Fusarium genus can produce mycotoxins that cause serious health problems on humans and animals. While Fusarium produces in pre-harvest period, Penicillium and Aspergillus species produces in exsiccation and post-exsiccation periods and creates toxins.

All mycotoxins are classified as fungal-originated. Yet, not every toxin produced by them are classified as mycotoxins. Some secondary metabolites of fungi can be substances that affect human health positively. For instance, fungal products that are produced by Penicillium species and that have toxic effects on bacteria are called antibiotics; while fungal products that are toxic to flora are called as phytotoxins (3).

The most-commonly known characteristic of mycotoxins is called “carry-over”. Therefore, in case of consumption of toxic feeds by animals and consumption of products

obtained from these animals (meat, milk, egg) can cause infection to humans. In a study conducted in 2003, *Fumonisin* accumulation has been revealed in the body tissues of animals which has been oral-fed of 100 mg FB1/animal Fumonisin per day during 11 days (Meger ve Ark,2003). This finding is an indicator of Fumonisins to be transferred through animals. It is known that mycotoxins have carcinogenic, teratogenous, tremogenous, hemorrhagic, dermatic, hepatotoxic, nephrotoxic, neurotoxic effects on humans (4).

Exposure of nutrition to mold happens in the following way.

Direct Exposure: Mycotoxins contamination occurs in such nutrition as ; soy beans, hazelnut, peanut, walnut, pistachio, almond, sunflower seed, cotton seed (oily seeds), bread, fruits, meat products that are ripen by natural molds, cheese and spices.

Indirect Exposure: Indirect contamination occurs through usage of additive products or raw materials that are contaminated by mycotoxins, in the production. Following examples can be given for the indirect contamination: Usage of patulin contaminated fruits in the production of fruit-juices, usage of aflatoxin contaminated figs in the production of dried-figs and smashed figs, usage of peanuts in the production of peanut butter and derivatives (5).

Carry-Over: Carry-over is defined as the characteristic of nutrition to be contaminated to mycotoxin. As farm animals consume feeds contaminated to mycotoxins, they digest the mycotoxins and egest the most of them through urine and stool. Yet, these metabolites can accumulate in body tissues (3).

In addition to that, mycotoxin contaminated products can cause serious economic damages. Contaminated nutrition can lose their economic values. The Food and Agricultural Organization (FAO) states that 25% of all globally produced products are contaminated by mycotoxins and they cause economic lose.

2.1.1. Aflatoxins

Aflatoxins (AF) are secondary metabolites of *Aspergillus flavu*s and A. *parasiticus molds* (6). It is commonly found in fruit juice, grain, milk and milk products.

When exposed to ultraviolet light, AFB1 and AFB2 gives blue florescent while AFG1 and AFG2 gives green florescent. AFM1 and AFM2 with less biological activity is found in the cattle which is fed with AFB1-AFB2 contaminated products. In terms of toxic effect, they rank as follows: AFB1 > AFG1 > AFB2 > AFG2 (7). These toxins cause carcinogenic, teratogenic and mutagenic complexities on humans and animals (8).

Among the toxic impacts of AF's, hepatotoxicity, hepato-carcinogenicity , nephrotoxicity, teratogenicity, inclination to be sick driven from weakening of immune system, growth arrest and decline of benefit gained from food stuff can be given (9).

Experiments on laboratory animals revealed that exposure to Aflatoxin causes liver cancer. Therefore, International Agency for Research on Cancer (IARC) evaluates "Group I" as carcinogen. As such, AFB1 is most commonly known human liver carcinogen (10).

2.1.2. Ochratoxins

Ochratoxins is the mycotoxin that are produced by mold species of *Aspergillus and Penicillium*. Molds that creates Ochratoxin are as follows: *A. ochraceus, A. malleus, A. sulphureus, P. verrucosum* and *P. palitans.* As Ochratoxin A is known to be a renal carcinogen which is found on grain and grain products, its nephrotoxic effect is proved on rats (11).

Clinical picture that constitutes of Ochratoxins is called "Ochra-toxicosis" . OTA causes destruction in renal cells and lesion accumulation in kidneys (12).

OTA leads to serious health complications on humans through DNA damages, protein synthesis inhibition, gluconeogenesis, oxidative phosphorylation damage in mitochondria and hemophilia. In addition to those, it can also cause renal tubule necrosis (13).

Some precautional and conservational approaches are commonly applied to scatter the impacts since fungi reproduction which produces ochratoxin on stored products cannot be prevented. Some of those are; careful control over contamination, usage of antagonists in case of exposure in human body, corroding via heat and radiation (14).

2.1.3. Trichothecenes

Trichothecenes are the secondary metabolites of some fungi as *Trichothecium, Fusarium, Cephalosporium, Stachybotry, Kerticimosporium, Cephalosporium* and *Cylindrocarpen.*

Among the most important mycotoxins in this group are Diacetoxyscirpenol (DAS), Deoxynivalenol (DON), Nivalenol (NIV), T-2 and HT-2. Toxicity ranking of these in a descending order is as follows: T-2 toxin > DAS > DON > NIV. DON is a mycotoxin that is most commonly found in food products (15).

Trichothecenes causes "Alimentary Toxic Aleukia" (ATA), akakabi-byo and skibo-trichosis diseases. In addition to those, they are also known to create hematological disorders,thrombocytopenia, leucopenia and agranulocytosis.

The most toxic trichothecene that is known is T-2 toxic. It affects the immune system, causes formation of free radical molecules, degradation of leucocyte and disorders in blood cells.

DON leads to acute exposed anorexia and emesis. IARC classifies "Group III" as carcinogen. ATA that is caused by trichothecenes is characterized by skin toxicity and bone marrow diseases (10).

2.1.4. Zearalenon

Zearalenone (Zen) is produced by *Fusarium* type fungi. It is commonly found in grapes, maize and high moisture straw. Zen is the estrogenic mycotoxin. Genital problems have been reported in animals exposed to Zen (11).

Risky products that create zearalenon toxin are as follows: maize and products containing corn, cereals, corn beer, bread, walnut and animal feeds.

Zearalenone can affect the digestion of dairy products when taken with forage of 1.3 mg/kg. Therefore, it can be found in raw, pasteurized, condensed milk, milk powder, soft or hard cheese. This toxin is not inactivated by pasteurization, boiling, freezing, cooling and cooking (Erol, 2007; Van, 1983; Aydın and friends, 2004). It is also known to have serious toxic effects on the liver (16).

2.1.5. Patulin

Patulin is the mycotoxin that are produced by some fungi as *Penicillium, Aspergilllus and Byssochlamys*. It is commonly found in apple, apple juice or concentrated apple. Patulin has toxicity for many living being. Additionally, it has also been reported to be carcinogenic (17).

Patulin is important for public health because it is toxic, mutagenic, cytotoxic and carcinogenic. It causes edema, connective tissue inflammation, rise of the blood sugar level and hypertension in human. Nausea, vomiting and stomach disorders are observed when taken orally. It may cause obstruction in the kidneys, degeneration in urinary tract and oliguria (Tunail, 2000).

2.2.FUMONISIN

Fumonisin is one of the most important mycotoxins. It was first isolated from Fusarium verticillioides which is the most commonly reported fungal species in 1988. Fumonisins are produced by several Fusarium species such as *F. verticillioides, F. proliferatum and F. Nygamai* (19) (Tablo 1).

Recently approximate 28 different Fumonisin are discovered. However, the most widely known is Fumonisin B1 (FB1). Fumonisin B1 and B2 are also known as the most toxic fumonisins.

FB1 is the mycotoxin that is the most prevalent member of a family of toxins, known as Fumonisins. It constitutes 70-80% of the total fumonisins produced in contaminated feed and the cultures of *F.verticillioides* (Ross and others, 1992). Additionally, FB2 constitutes 15-20% of the total Fumonisin and FB3 constitutes 3-8% of the total Fumonisins in the nature (Jackson and Jablonski, 2004).

There are many Fusarium species which can produce fumonisins. The metabolites produced by these species may also have differences between each other.

Table 2. Fumonisin-producing Species (Production of Fumonisin Analogs by *Fusarium* Species Rheeder and othr., 2002)

***Fusarium* spp.**	**Fumonisin türevleri**
F. verticilloides MP-A	FA_{1-3}, FB_{1-5}, iso-FB_1, FAK_1, FBK_1, $FC_{1,4}$, FP_{1-3}, PH_{1a-b}
F. sacchari MP-B	FB_1
F. fujikuroi MP-C	FB_1
F. proliferatum MP-D	FA_{1-3}, FB_{1-5}, FAK_1, FBK_1, FC_1, FP_{1-3}, PH_{1a-b}
F. subglutinans MP-E	FB_1
F. subglutinans MP-	FB_1
F. thapsinum MP-F	FB_{1-3}
F. anthophilum	FB_{1-2}
F. globosum	FB_{1-3}
F. nygamai MP-G	FA_{1-3}, FB_{1-5}, FAK_1, FBK_1, FC_1, FP_1, PH_{1a-b}
F dlamani	FB_1
F. napiforme	FB_1
F. pseudonygamai	FB_{1-2}
F. andiyazi	FB_1
F. oxysporum	$FC_{1,3-4}$, N-acetyl-FC_1, N-acetyl-iso-FC_1,OH-FC_1, N,acetyl-OH-FC_1
F. oxysporum var. *redolens*	FB_{1-3}
F. polyphialidicum	FB_1

Factors affecting the combination level of agricultural products with Fumonisin spp. and Fumonisins are as follows;

- Seed Type
- Climate
- Agronomic Practices
- Plant stress
- Soil Moisture Content
- Maximum temperature
- Nutrient content of soil
- Competitive Microflora

Foods and production stages can change the microflora that can be accommodated. This situation can lead to contamination. Seed variety is one of the most important points for the microbial structure to be kept by the plants. Because there is a

special microbial structure for each plant. At the same time, temperature and climatic conditions may be different in terms of microorganisms on foods. Fumonisin amounts of foods in regions with high temperature and humidity are higher than in other regions.

Fumonisin formation amounts vary in different nutriments. Different contamination percentages may occur from species to species (Table 2).

Table 3. Substrates which some molds are isolated from and Fumonisin-forming potentials

Fusarium spp.[1]	Substrat	Fumonisin üreten suş sayısı/toplam suş sayısı			Kaynak
		FB_1	FB_2	FB_3	
F. verticilloides	Arpa	2/3	2/3	-[3]	Sala ve diğ., 1994
	Arpa	5/5	5/5	-	Visconti ve Doko, 1994
	Buğday	1/4	1/4	-	Sala ve diğ., 1994
	Buğday	6/6	6/6	-	Visconti ve Doko, 1994
	Mısır	40/112	20/112	-	Sala ve diğ., 1994
	Muz	8/16	4/16	-	Jiménez ve diğ.,1997
	Sorgum	1/7	0/7	-	Sala ve diğ., 1994
	Tıbbi bitkiler	6/6	6/6	-	Rizzo ve diğ., 2004
F. profileratum	Buğday, kara buğday	4/5	-	-	Nelson ve diğ., 1992
	Muz	6/9	3/9	-	Jiménez ve diğ.,1997
	Mısır bazlı hayvan yemi	4/9	-	-	Nelson ve diğ., 1992
	Mısır püskülü	2/2	-	-	Nelson ve diğ., 1992
	Yer fıstığı, toprak, toprak döküntüsü[2]	8/12	-	-	Nelson ve diğ., 1992
	Tıbbi bitkiler	1/1	1/1	-	Rizzo ve diğ., 2004
	Sorgum	1/1	1/1	-	Visconti ve Doko, 1994
	Tavuk yemi	86/86	-	-	Labuda ve diğ., 2005
F. subglutinans	Mısır	0/20	-	-	Nelson ve diğ., 1992
	Mısır	1/6	1/6	-	Sala ve diğ., 1994
	Muz	0/3	0/3	-	Jiménez ve diğ., 1997
	Sorgum	0/1	-	-	Nelson ve diğ., 1992
	Tavuk yemi	0/16	-	-	Labuda ve diğ., 2005
F. globosum	Mısır	17/17	15/17	14/17	Sydenham ve diğ., 1997
F.nygamai	Darı	4/14	-	-	Nelson ve diğ., 1992
	Toprak, darı[2]	1/2	1/2	-	Thiel ve diğ., 1991
F. napiforme	Sorgum	1/11	-	-	Nelson ve diğ., 1992
	Sorgum	0/1	0/1	-	Thiel ve diğ., 1991
	Darı	0/15	-	-	Nelson ve diğ., 1992
F.dlamini	Toprak	5/9	-	-	Nelson ve diğ., 1992
F.anthophilum	Mısır, tahıllar, toprak[2]	3/17	-	-	Nelson ve diğ., 1992
	Yulaf	0/1	0/1	-	Thiel ve diğ., 1991

[1]:Fumonisin oluşturma özellikleri mısıra aşılanarak incelenmiştir.
[2] : Farklı kaynaklardan izole edilmiştir.
[3]: Veri bulunmamaktadır.

2.2.1. Effects of Fumonisin on Health

It has been reported that Fumonisin causes brain cell damage (LEM) and sudden death in horses, liver problems and cancers in mice, pulmonary edema (PPE-Parcine Pulmonery Edema) and cardiovascular diseases in pigs (21). Also, it is hepatotoxic, nephrotoxic and immunosuppresant depending on animal species (Nelson and others, 1992). In general, liver and kidneys are the main target organs. Subcutaneous injection during an experiment or very high dose may also cause neurotoxic effects (EHC, 2000).

After the discovery of the fumonisins, mass death of animals has been occurred in the production areas in ABD. PPE and LEM have been observed in a large scale especially in animals feeding on contaminated corn and corn stalk. This is also due to the 'carry over' feature of the Fumonisins. Deaths occurred after 3-4 days in horses exposed to high Fumonisin. Studies have shown that there is a positive correlation between animal deaths and contaminated food consumption (Dejardins, 2006).

Fumonisin not only affects kidney, liver and brain functions. It can cause damage or deterioration in other tissues and organs. According to one study, to examine the effect on the fecal microbiota, 12 mg / kg of FB1 per day were given to pigs (n=6). The fecal values of these subjects are checked after 0, 8, 15, 22, and 29 days of exposure. A control group of six piglets received a diet free of FB1. In conclusion, exposure to FB1 causes to decrease the diversity index, and shifts and constrains the structure and the composition of the bacterial community. And also, it created toxicity (22). There is a need for new studies on the destruction of fumonisins to other tissues and organs.

Fumonisins are low molecular weight. Taking roles as receptors of important molecules in metabolism, they affect nucleic acids, protein synthesis, enzymes and hormone activity. Fumonisins have been shown to cause some important changes in lipid metabolism. Fumonisins affect the sphingolipid mechanism which acts as a structural component in the membranes of animal and plant cells. Phytotoxic mechanism negatively affects protein synthesis. It destroys the cell membrane;hence it causes damage to the cell. It has been reported that the mechanisms of action are inhibition of protein kinase and serin/treonin phosphate enzyme in animals (Riley, 1998).

A study conducted in South America on fumonisin have shown that there is a significant association between the amounts of fumonisin in foods and cancer(23). This linkage associates with cell destruction and disruption of receptor functions.

Because of all these effects, the International Agency for Research on Cancer (IARC) defined Fumonisin B1 as a 2B class human carcinogen (24). Although there are no serious studies on people, it shows that the situation is also serious for people considering the animal deaths and sudden deaths occurring in horses. Therefore, the maximum tolerable daily use of Fumonisin was determined as 2 µg/kg.

Table 4. Mycotoxins and Health Problems caused by (1)

Mycotoxin	Species	Toxic effects	Effects on immune system
Aflatoxins	Humans, all other mammals, birds, fish	Hepatotoxicity, bile duct hyperplasia, intestinal and renal hemorrhage, liver tumors	Reduced lymphoproliferation, delayed hypersensitivity, phagocytosis, antibody formation against T-dependent antigens, increased infections
Fumonisins	Human, pig, horse, mouse, rat,	Pulmonary edema in swine, leukoencephalomalacia in horses, liver and kidney damage, esophageal and hepatic cancer in humans (?)	Decreased lymphocyte blastogenesis; decreased antibody titers after antigens or vaccines. Decreased splenic and thymic cellularity and interleukin-2 production (in female mice)
Ochratoxin A	Human, swine, dog, duckling, chicken, rat	Nephrotoxicity, enteritis, liver damage, teratogenesis, renal carcinogenesis	Decreased phagocytosis, cellular depletion of lymphoid organs, transient immunostimulation
Patulin	Birds, mammals (cat, rabbit, cattle)	Lung hemorrhage, capillary damage, convulsions, brain edema, carcinogenesis (mammals and birds)	Reduced DNA synthesis in lymphocytes, decreased peripheral leukocytes, altered distribution of lymphocyte subpopulations
Trichothecenes	Human, pig, cattle, chicken, horse, rat, mouse, dog	Vomiting, diarrhea, bleeding, dyspnea, itching, rash, blisters, leukopenia	Increased infectivity, decreased lymphoproliferation and antibody formation, decreased macrophage cytokine production

2.2.2. The Formation of Mycotoxin and Reducing Its Effects

Mycotoxin exposure affects many people and animals around the world. In order to prevent contamination of mycotoxins and the consumption of contaminated foods, people's awareness should be increased.

Methods for reducing mycotoxin exposure can be discussed as follows;

a) Reducing the water activity value (aw) to less than 0.60 depending on the preservation methods for nutriments

b) inhibition of oxygen uptake

c) Proper maintenance

d) Disintegration of mycotoxins in nutriment and feed by physical-chemical-biological methods

e) Not to feed moldy foods to animals

f) Reducing the toxicity of mycotoxins

g) To control mycotoxin in concentrated feeds given to animals

h) Continuity of traceability of mycotoxins in terms of human health and national economy

ı) Consumption of food and feed exceeding legally accepted limits should be prohibited.

The production stage is the area where molds producing mycotoxin are most frequently contaminated. For this reason, manufacturers should use various methods and apply new techniques to prevent contamination.

Another important issue is preventing the consumption of contaminated foods. As a matter of fact that not to waste foods with high economic value and the desire to evaluate these foods causes people to consume them. At the same time,foods that are not stored under suitable conditions and which are not suitable for use are consumed in the domestic life.

In some cases, contaminated food that is not suitable for human health is used as animal feed to utilize. However, most of mycotoxins can cause accumulation in animal meat or milk and may have a negative impact on human health.

Physical techniques, natural or synthetic chemicals and biological methods are used in studies to reduce the risks of contamination and toxins.

Use of non-contaminated seed with fungi, control of insects and diseases, adequate inoculation, protection from drought, rapid harvesting when the product grows mature, the use of harvesting techniques that minimize mechanical damage can prevent fungal placement and aflatoxin formation. These methods need to be explained with detailed trainings for each individual from the producer to the end consumer (25).

2.3. Importance of Peanuts

Thanks to the macro and micro nutrients they contain, oil seeds which are an important factor in human and animal nutrition are also used as an important raw material in the industry. Due to the inexpensive and insufficient production of animal oil, vegetable oils are used for alimentation (Arıoglu, 2009). Peanut seeds which contain oil, protein, carbohydrate, vitamin and mineral materials are great importance for human and animal nutrition (MOE, 2012). Peanut oil is used in fish preservation, making biscuits, pastry, candy and soap as well as cooking. The defatted peanut pulp is a valuable feed. Peanut shells are used in the production of chipboard, as a feed filler, in mushroom cultivation, as a fuel, as a filler in wood-making, in the production of artificial coal, as forage in cattle breeding, as mulch in poultry farming (Woodrof, 1973). (Woodrof, 1973)

One of the most important of these oilseeds is peanuts. Peanut (Arachis hypogaea L.) which is a native South American legume is produced due to its nutritive value and its fat content. Generally, it is used as a snack but is also used in oil industry. The production in our country is increasing every year. It is produced especially in our southern regions. It is widely consumed as a snack such as roasted, non-roasted and with various types of coating (honey, sesame, corn fed) in the world (4)

Turkey ranks first in the world according to the production of approximately 600 thousand tons of nuts in 1997-2001 (26). As a consequence, nuts production is a serious economic income channel in Turkey.

Peanuts are cultivated in a total of 15 provinces as a majority in the Mediterranean and the Aegean refion in Turkey.These are Osmaniye, Adana, İçel, Aydın, Kahramanmaraş and Muğla (Gerçekcioğlu and Guneş, 1995). In 2000, China ranked first with 43.7% in the world followed. By İndia (17.7%), Nigeria (%8) and USA (4.3%). As it can be understood, more than half of the world production of peanuts. On the basis of continents, peanuts are supplied at the rate of 70% in Asia, 20% in Africa, 8% in N.America and 2% in the S.America. Turkey has the little importance in world trade. The share of Turkey in exportation is less than 1%. Exports of unshelled peanuts in Turkey as of 1999 is 270 tons while 112 tons of shelled peanuts are exported (Annonym, 2000).

One of the most important issues for the production and consumption of peanuts is to ensure that no adverse health effects are experienced in human and animal health. One of the most important points in this regard is to ensure food safety and to deliver the products safely to the final consumer. Microorganisms that may occur on the nutrients and their toxic structures can affect human and animal health negatively. In peanuts, during the harvesting phase, mycotoxins can be formed during the drying and storage and they can reach the levels that may threaten the health of the consumer.

3.MATERIAL AND METHOD

3.1. MATERIAL

20 g samples of peanuts from different parts of Istanbul were collected. These peanuts are sold in the open. There were total 45 samples. They were brought to the laboratory under sterile conditions in sterile packages and were kept at 2-8 ° C until the day of the assay.

3.2. METHOD

The study was carried out in Yeditepe University Nutrition and Dietetics Department and Medical Microbiology Department Laboratory of Medical Faculty. The following tools were used;

- Laminar workbench: For non-contaminated processing of samples.
- Shaker incubator: for homogenization.
- Elisa waher (Biotech 50): for washing in Elisa.
- Elisa Reader (Beckman): for testing the Elisa results.
- Fume hood: for the preparation of chemicals.
- Micro incinerator: for sowing under sterile conditions.

3.2.1. Detection of Fumonisins by Elisa Method

The amount of Fumonisin in the collected samples was determined by Elisa method by using AgraQuant Fumonisin (0.25-5.0 ppm) kit. This kit is a competitive direct Enzyme-Linked ImmunoSorbent Assay. Fumonisin released by 70% methanol is discovered by the wells covered with enzyme-conjugate. The manufacturer's recommendations is considered as a procedure. Firstly, the samples were prepared according to the following procedure for using in Elisa method.

1. 20 g of the samples were weighed one by one for analysis.
2. The methanol-distilled water mixture was then prepared in the ratio of 70/30 in fume hood. 100 ml of this mixture was added to each sample.
3. Afterwards,the samples were shaken in a shaker incubator for 3 min.
4. At the next stage, samples were filtered by using Whatman No: 1 filter paper.
5. In the final stage, 50 µl of elution was used in Elisa test.

In order for the Elisa test to be accurate, the kit components were first reached to room temperature. Since the standards contained in the kit are ready for direct use, no reconstitution has been carried out.

The following procedure was applied for the Elisa test.

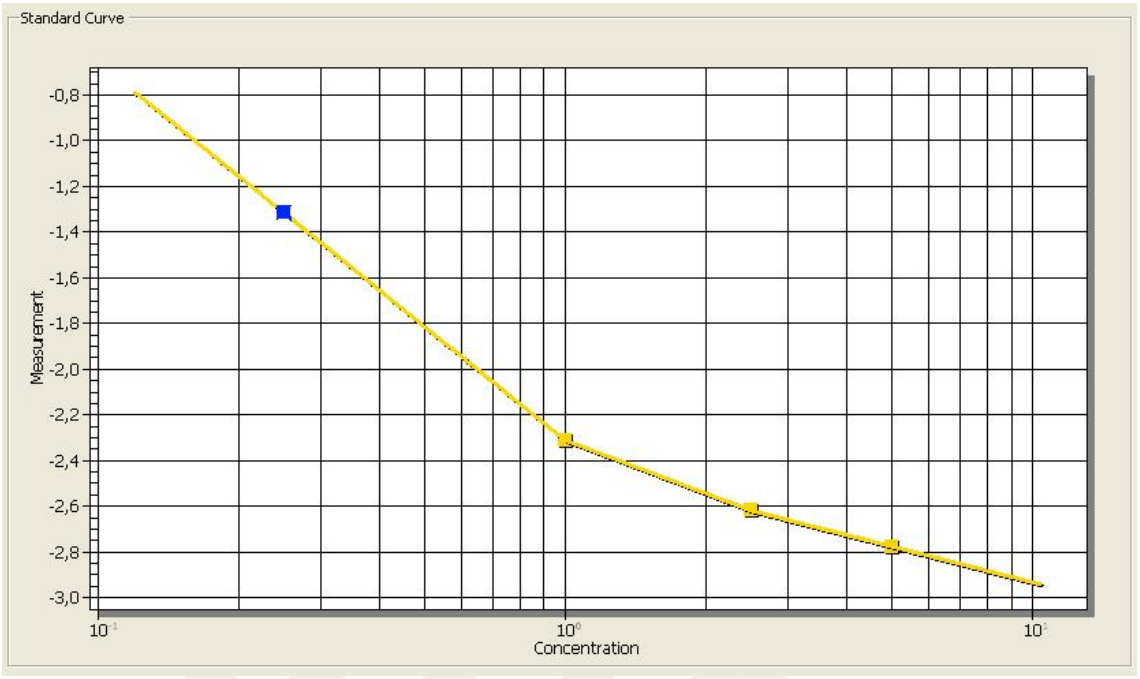
1. In the first stage, the samples were diluted in the ratio of 1/20. For example, for a 50 µL sample, dilution was made to 950 µL distilled water.

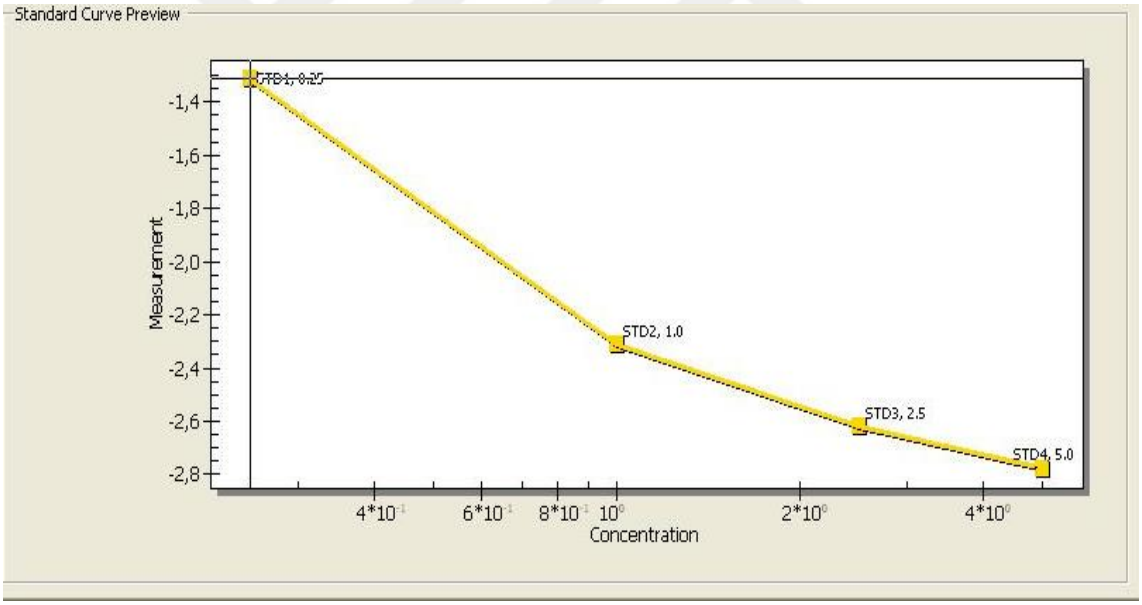
2. Dilution plates include samples diluted with 200 µL of conjugated. 100 µL of these were pipetted. The plate was then carefully shaken.
3. In the next step, 100 µL was transferred from the dilution plate to the antibody coated plate by means of the multichannel pipette.
4. The plate was incubated at room temperature for 10 min.
5. At the end of the incubation, the plate was washed 5 times in an automatic Elisa washer. This device minimizes contamination between samples and false positives due to manual washing.
6. In the next step, 100 µL of substrate was added to all the wells by means of an automatic pipette.
7. They were incubated for 5 min at room temperature and in the dark.
8. At the last stage, 100 µL stop solution was added and Elisa test was completed.
9. The Elisa plate was then read in the Elisa reader in a 450 nm filter (by 630 reference filter) and absorbance values were taken. In addition, Fumonisin values of the samples were determined quantitatively by drawing a standard curve graph using the device's program.

3.2.2. Detection of Culture

Sabouraud 4% Dextrose Agar (SDA) (Merck, Germany) was used for culture detection. The peanut samples were seeded in SDA medium and incubated for 7-10 days. At the end of the incubation, each colony was steeped in SDA and allowed to incubate for 7-10 days. Colonies obtained at the end of incubation were evaluated by microscopy with preparates prepared with Lactophenol Cotton Blue.

Quality control of the study was performed. The results are as follows.





4.RESULTS

In this study, 45 peanuts each of which was 20 grams sold from different locations of Istanbul were prepared and examined for ELISA test method. At the end of the study, the presence of mycotoxin in all 45 samples was determined The peanuts tested were evaluated according to the Communiqué on Maximum Limits of Contaminants in the Turkish Food Codex Food Items. None of the 45 peanut samples collected showed the presence of fumonisin above the limits specified in the notification

Table 5. Maximum amounts of Fumonisin that nutriment can contain according to Turkish Food Codex

Gıda Maddesi	Maksimum limit ($FB_1 + FB_2$) (µg/kg)
2.6. FUMONİSİNLER [15]	
2.6.1. İşlenmemiş mısır (ıslak öğütülecekler hariç) [13]	4000
2.6.2. Doğrudan insan tüketimine sunulan mısır, dorudan insan tüketimine sunulan mısır bazlı ürünler	1000
2.6.3. Mısır bazlı kahvaltılık tahıllar ve mısır bazlı çerez	800
2.6.4. Bebek ve küçük çocuk ek gıdaları (işlenmiş mısır bazlı olanlar) [3]	200
2.4.5. 500 mikrondan büyük eleklerden geçirilerek üretilen mısırın kabaca öğütülmesinden elde edilen küçük parçalar ve mısır irmiği (GTİP 1103 13) veya mısırdan elde edilen pelleter (GTİP 1103 20 40) ve doğrudan insan tüketimine sunulmayan 500 mikrondan büyük eleklerden geçirilerek üretilen mısır veya mısır ürünlerinin kabartılması veya kavrulması suretiyle elde edilen gıda maddeleri (GTİP 1904 10 10)	1400
2.4.6 500 mikrondan küçük ve eşit eleklerden geçirilerek üretilen mısır unu (GTİP 1102 20) ve doğrudan insan tüketimine sunulmayan 500 mikrondan küçük ve eşit eleklerden geçirilerek üretilen mısır veya mısır ürünlerinin kabartılması veya kavrulması suretiyle elde edilen gıda maddeleri (GTİP 1904 10 10)	2000

Table 6. The result of the study is shown in the table.

Sample No	Results (µg/20 gr)	Results (µg/kg)		Sample No	Results (µg/20 gr)	Results (µg/kg)
1	0,272	13,6		**21**	0,353	17,65
2	0,286	14,3		**22**	0,308	15,4
3	0,305	15,25		**23**	0,248	12,4
4	0,437	21,85		**24**	0,263	13,15
5	0,298	14,9		**25**	0,307	15,35
6	0,309	15,45		**26**	0,306	15,3
7	0,397	19,85		**27**	0,299	14,95
8	0,265	13,25		**28**	0,466	23,3
9	0,281	14,05		**29**	0,353	17,65
10	0,274	13,7		**30**	0,335	16,75
11	0,33	16,5		**31**	0,303	15,15
12	0,447	22,35		**32**	0,332	16,6
13	0,513	25,65		**33**	0,354	17,7
14	0,342	17,1		**34**	0,342	17,1
15	0,339	16,95		**35**	0,388	19,4
16	0,312	15,6		**36**	0,364	18,2
17	0,323	16,15		**37**	0,423	21,15
18	0,35	17,5		**38**	0,315	15,75
19	0,335	16,75		**39**	0,362	18,1
20	0,364	18,2		**40**	0,371	18,55

Limit: 1000

µg/kg

5.DISCUSSION AND CONCLUSION

45 peanut samples for sale in various regions of Istanbul were tested by ELISA for the content of fumonisin and evaluated according to the Communiqué on Maximum Limits of Contaminants in Turkish Food Codex Foods. The results of the peanut samples tested in the study were negative. The values found in our research are suitable for fumonisin.

Elisa test method was used in our study. However, there are many different methods and kits applied in scientific studies.

In the determination of mycotoxins, different methods such as thin layer chromatography, gas chromatography, high pressure liquid chromatography (HPLC) are used. Also mass spectrometer is utilized for the study. Chromatography is a method commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed. Liquid / liquid, solid / liquid, solid / gas or liquid / gas phase chromatographic methods have been developed according to the constant and mobile phase used in chromatography is liquid, solid or gas. Solid / liquid phases are used in Thin-layer chromatography. Commonly used analytical methods for the identification of mycotoxins are immunochemical methods such as high performance liquid chromatography (HPLC), fluorescence detection, thin layer chromatography (TLC), and enzyme-linked immunosorbent assay (ELISA). A cleaning step is required for both HPLC and TLC methods using solid phase extraction or immunoaffinity column. ELISA test kits are preferred as high entry assays with lower sample volume requirements and less sample cleaning procedures than conventional methods such as TLC and HPLC. They are fast, simple, specific, precise and portable. It has also become the most common method for the detection of mycotoxins in foods and nutrients. However, since the produced antibodies frequently cross-react to compounds like mycotoxins, a comprehensive study which is conducted for the accuracy and precision of the ELISA method cannot elicit clear data.

When we checked the studies in the literature, Elisa (enzyme-linked immunosorbent assay) test method was used in a study on 95 corn samples taken from local producers in Ethiopia in 2013. According to the results of the study, all samples were reported to be contaminated with Aspergillus and Fusarium species. Total fumonisin values in the samples were found between 907 and 2041 7g / kg. Fumonisin values are reported to be lower in fresh samples (27).

According to a study conducted on 108 samples sold in different markets in Iraq; Fusarium species in samples were investigated. According to the results of the study, 28 fungi were isolated according to their morphological characteristics. These were F. proliferatum (12), Aspergillus niger (8), Aspergillus flavus (5), and Penicillium sp. (3) (28). (3)). In this study, it was determined that the nuts were contaminated. If the storage conditions are not observed, these foods are expected to form mycotoxins in the near future.

In a study conducted on corn products sold in Poland, 106 samples were collected. HPLC method was applied and Fumonisin amounts of samples were investigated. 34.9% of the samples were contaminated with Fumonisin (FB1 and FB2) and 45.3% were contaminated with FB1 and 17.0% by Fb2. 6342 mg / kg fumonisin was detected in one of these samples.

In 2010, 25 canned corn, corn flour and corn flakes were collected from the markets. According to the Elisa method, the amount of Fumonisin in 6 of 25 corn flour samples was found to be higher than the levels (2 mg / kg) indicated by Turkish Food Codex (TFC). The amounts of fumonisin detected in corn flakes and corn canned were within acceptable limits (30).

According to the study conducted in the Aegean region in between 2003 and 2004, 115 fig samples were collected from 7 different regions in the drying stage and fumonisin amounts and contaminated species were investigated. According to the results of the research, the number of Fusarium on the samples was determined more than the number of A.section Nigri and A. fusion and Penicillium in both years. Fusarium contamination was determined in 88 of 115 samples. Contamination on food is an indication that deterioration may increase in the future.

According to another study conducted in our country, Fumonisin levels in cereal and cereal products were investigated. Fumonisin analysis of 57 grain and cereal products are all corn and corn based products.Additionally, Fumonisin values were found in 42 of them.And also 3 of them had Fumonisin level that was above the legal limit (31).

AFB1 (5.67 ± 4.75 µg / kg) was detected in 80% of the corn samples obtained from the 51 corn samples collected from four provinces of Iran (Fars, Kermanshah, Khuzestan, Mazandaran) and Mazandaran Provinces (18). In a study conducted on 70 wheat flour and wheat samples tested, 17 samples (24.28%) has been covered with aflatoxin and ochratoxin. The ELISA method in human food samples showed that 2% of the samples were contaminated with fumonisin and 3% were contaminated with aflatoxin (33). 21 (48%) out of 43 cereal samples in India were found to be contaminated with total Fumonisin (34).

In the light of the study, similar data are obtained in the literature. Many factors can be effective such as Fumonisin values, production, humidity, climate, storage conditions, how long the product waits on the shelf, the product is prepared under which conditions. Due to our suspicion about food safety, we think that new studies should be carried out in different food groups. We believe that the use of packaged products and the preference of licensed enterprises and brands by the Ministry of Food, Agriculture and Livestock will be a more accurate way for food safety.

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