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Determination of Total Aflatoxin in Packaged and Unpackaged Black Pepper Samples

MASTER THESIS

Nur Sena TOKDEMİR

İstanbul-2017

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SUPERVISOR

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TEZ ONAYI FORMU

Kurum : Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü

Program : Beslenme ve Diyetetik Yüksek Lisans

Tez Başlığı : "Kapalı ve Açıkta Satılan Karabiber Örneklerinde ki Toplam Aflotoksin Miktarının Kıyaslanması"

Tez Sahibi : Nur Sena TOKDEMİR

Sınav Tarihi : 02/05/2017

Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Yüksek Lisans Tezi olarak kabul edilmiştir.

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun ..18.../..05.../..2017.. tarih ve ..2017/07-10....sayılı kararı ile onaylanmıştır.

İmza

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Sağlık Bilimleri Enstitüsü Müdürü

DECLARATION

I hereby declare that this thesis contains 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.

Date

Signature Name Surname

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor Assit. Prof. Dr. İskender KARALTI for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

I would especially like to thank my father Yusuf TOKDEMİR for his encouragement and support.

I would also like to thank to the staff of the microbiology laboratory of Yeditepe University.

I would like to extend my thanks to all the instructors of Department of Nutrition and Dietetics at Yeditepe University for their contribution to my master education. They always have been kind and supporting individually.

Finally I would like to thank my family and friends for their encouragement and moral support.

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ABBREVIATIONS

% : Percentage

g : Gram

ml : Mililitre

mm : Milimeter

°C : Celcius

Ppb :Parts per billion (µg/kg)

HPLC : High Performance Liquid Chromatoghraphy

ELISA :Enzyme Linked Immunosorbent Assay

ABSTRACT

Tokdemir N. (2017). Determination of Total Aflatoxin in Packaged and Unpackaged Black Pepper Samples. Yeditepe University, Institute of Health Sciences, Department of Nutrition and Dietetics, MSc thesis. İstanbul.

Aflatoxins cause great economic loss due to the effects on human and animals. Here, farmers, stock, suppliers, traders, consumers in the chain from production to consumption are damaged and even reach the national dimensions. Food and feed containing aflatoxin is not allowed to be consumed at levels higher than the limits agreed in the legal regulations. In the production phase, the aflatoxin problem with increasing or decreasing dimensions due to seasonal conditions, other cultural measures to improve quality should be applied, as well as other methods will provide a definitive solution.

This study was carried out with the aim of determining the total amount of aflatoxin in the black pepper samples presented in sales in the city of Istanbul (Turkey) packed and unpacked, which are obtained from the market.

20 different samples of unpacked black pepper were obtained from the Spice Bazaar and from different spice stores in Eminönü. The samples prepared in 25 g packages were examined in the laboratory in such a way that they not contaminate with each other. Samples of 16 packaged black pepper were taken from the spice stores and supermarkets around Istanbul and examined in the laboratory in 25 gr samples.

A Romer brand AgraQuant Total Aflatoxin Elisa Kit (USA) was used to determine the total amount of aflatoxin in all samples. This kit works with the competitive Elisa method. ELISA is one of the very fast, simple and special methods used to analyze mycotoxins in food. All samples were prepared using Romer brand Aflastar columns suggested by the kit manufacturer and the samples obtained were used in ELISA test. The total amount of aflatoxin should be below 10 ppb according to the Turkish Food Codex (TGK) and the US Food and Drug Administration (FDA). In our study, the levels of aflatoxin in the black pepper samples which were sold as open and packed were determined and one sample exceeded the limits. This sample with 10.49 ppb was found and the others were between 3.928 and 0.453 ppb. It is necessary to

work with HPLC to determine the precise aflatoxin weights of this sample which is above the limits and is at the limit value.

In our study, moulds were isolated from 8 out of 36 samples. *Aspergillus niger* in 2 samples, *Rhizopus spp.* in 2 samples and *Penicillium spp.* in 4 samples. *Aspergillus niger* (5%), *Penicillium spp*. (11%) and *Rhizopus spp*. ratio (5%) were isolated in the black pepper samples packed and unpacked.

Because aflatoxins are harmful to human health, it is absolutely necessary to analyze the mycotoxins before sale and especially for the products sold in the open, at certain intervals.

Key words: Total aflatoxin, ELISA, Turkey, Black pepper.

ÖZET

Tokdemir N. (2017). Kapalı ve Açık Olarak Satılan Karabiber Örneklerinde ki Aflatoksin Miktarlarının Belirlenmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik ABD, Master Tezi. İstanbul.

Aflatoksinler, insan ve hayvanlar uzerindeki etkileri nedeni ile büyük ekonomik kayiplara sebep olmaktadır. Burada uretimden tuketime kadar olan zincirde yer alan ciftci, besisi, tüccar, tüketici zarar görmekte hatta konu ülkesel boyutlara bile ulaşmaktadır. Yasal düzenlemeler doğrultusunda kabul edilen limitlerden daha yüksek değerlerde aflatoksin içeren gıda ve yemlerin tüketilmesine izin verilmemektedir. Üretim aşamasında, mevsimsel koşullara bağlı olarak boyutları artan veya azalan aflatoksin probleminde, kaliteyi iyileştirmeye yönelik bazı kültürel önlemler dışında kesin çözüm getirecek diğer yöntemlere de başvurulmalıdır.

Bu çalışma İstanbul ilinde (Türkiye) piyasadan elde edilen paketli ve paketsiz olarak satışa sunulan karabiber örneklerindeki toplam aflatoksin miktarının belirlenmesi amacı ile yapılmıştır.

20 farklı paketsiz karabiber örneği Mısır çarşısı ve Eminönü'ndeki farklı aktarlardan temin edilmiştir. 25 gr'lık paketler halinde hazırlanan örnekler birbiriyle kontamine olmayacak şekilde laboratuvarda incelenmiştir. 16 paketli karabiber örneği ise İstanbul çevresindeki aktar ve marketlerden alınarak 25 gr'lık örnekler halinde laboratuvarda incelenmiştir.

Bütün örneklerdeki toplam aflatoksin miktarını belirlemek için Romer marka AgraQuant Total Aflatoksin Elisa Kit (ABD) kullanılmıştır. Bu kit yarışmalı Elisa metodu ile çalışmaktadır. ELİSA gıdalardaki mikotoksinleri analiz etmek için kullanılan oldukça hızlı, basit ve özel yöntemlerden bir tanesidir. Bütün örnekler kit üreticinin önerdiği Romer marka Aflastar kolonları kullanılarak hazırlanmış ve elde edilen numuneler elisa testinde kullanılmıştır. Toplam aflatoksin miktarı, Türk gıda kodeksi (TGK) ve Amerikan Gıda ve İlaç Dairesi (FDA)' ne göre 10 ppb'nin altında olması gereklidir. Çalışmamızda açık ve paketli olarak satılan karabiber örneklerindeki aflatoksin düzeyleri belirlenmiş ve bir örnek limitlerin üstünde bulunmuştur. 10.49 ppb bulunan bu örnek dışındakiler 3.928 ile 0.453 ppb arasında saptanmıştır. Limitlerin

üstünde çıkan ve sınır değerde olan bu örneğin kesin olarak aflatoksin yükünün belirlenmesi için HPLC ile çalışılması gereklidir.

Çalışamızda 36 örnekten 8 tanesinde küf izole edilmiştir. 2 örnekte *Aspergillus niger* diğer 2 örnekte *Rhizopus spp* ve 4 örnekte de *Penicillium spp* dir. Paketli ve paketsiz olarak satılan karabiber örneklerinde izole edilen *Aspergillus niger* oranı (5%), *Penicillium spp* oranı (11%), *Rhizopus spp* oranı (5%) olarak tespit edilmiştir.

Aflatoksinler insan sağlığı için zararlı oldukları için mutlaka satış öncesi ve özellikle açıkta satılan ürünler için belirli aralıklara mikotoksinler açısından analiz yapılması gereklidir.

Key words: Total aflatoxin, ELISA, Turkey, Black pepper.

1.THEORTECAL BACKGROUND

1.1 Mycotoxins

Mycotoxins are toxic metabolism products produced by some moulds. Mycotoxin-producing molds include *Aspergillus, Penicillium* and *Fusarium*. Infection causes toxicoses (mycotoxicosis) at various levels in humans and animals receiving feed and nutrient. (1)

1.1.1 Discovery of Mycotoxins

First, at the beginning of 1960, mycotoxins were recognized by the outbreak of turkey x disease in England. Approximately 100,000 turkeys died due to peanut contaminated with *Aspergillus flavus* in their feed. Since then many other toxic compounds have been identified that are produced by filamentous fungi. It is known that more than 250 mushroom species have formed mycotoxin up to now (2).

Different moulds produce different mycotoxins. More than 400 mycotoxins were described up to now and 20 micotoxins were described as having high toxicity to humans and animals. The most known species are *AFs, ochratoxin (OTA), patulin (PAT), fumonisins (FBs), zearalenone (ZEN), trichothecenes,* and *Aspergillus, Penicillium, Fusarium and Claviceps* (2).

Mycotoxins may be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, immunosuppressive, and estrogenic. The severity of the effects depends on the amount taken in large quantities, the duration of exposure, the level of toxicity and the simultaneous takingl of different mycotoxins. Exposure to high levels of mycotoxins may cause acute toxicity and death of persons. When it is below the lethal level, it is seen that the immune system is decreased and the resistance against infections is decreased. When consumed at low rates for a long time, it was determined that vital organs caused chronic diseases and tumors (2).

1.2. Mycotoxins and Producer Fungus in Food

Among the major mycotoxin producers, *Alternaria* and *Fusarium* species belong to field moulds, *Penicillium* and *Aspergillus* belong to store moulds. Depending on the composition of each product, the moisture content, the climate conditions, and the types of moulds, types, proportions, and types and amounts of mycotoxins they develop on the product vary. When we examine the products of cereal and other grain plants (legumes) in terms of mould populations and mycotoxins to form an example, we encounter this spectrum (3).

The field moulds are the fungus that remain outside of the rust and burn factors, which are transmitted to the mature grains before the harvest. In this field, 70 moulds and 150 species were isolated. Among these; *Alternaria, Aureobasidium, Botrytis, Cladosporium, Helminthosporium, Fusarium, Stemphylium* and *Verticillium* are the dominant and important ones. The spores in the cones of the moulds are carried by the wind and water to grains, or the infected parts of the plant are contacted with the grains. Colour and image of contaminanted grains change, because of germination of spores and moulds, germination ability decreases, mycotoxins occur. These products are firstly infested with *Fusarium*, among the *Fusarium* toxins, deoxynivalenol, nivalenol, diacetoxysirpenol, HT-2 and T-2 toxins enter the group of tricothecenes. Also zearalenone and fumonosine can be synthesized. However, in a convenient storage, if the moisture content in the grains is dried on condition not exceed 13.5 - 14%, kept in 10-15 ºC in dry well-cleaned silos, contaminated field moulds and toxin formation is prevented. Furthermore, tricotheenes from the formed mycotoxins are metabolized and completely disrupted in storage conditions. As the product contained in the silo enters constant conditions, the mould spectras start to change. The transition fungus flora occurs before the storage moulds. For example, *Epicoccum, Chatemium, Nigrospora, Rhizopus, Papullaria, Fusarium nivale, Trichothecium roseum* genus and species occur. In long-term stored garins of cereals and legumes, *Eurotium, Aspergillus* and *Penicillium* species become dominant. In Table 1, Important filamentous fungi and mycotoxins seen in forage crops and silages were classified (3).

Table 1: Important filamentous fungi and mycotoxins in forage crops and silages (4).

Filamentous fungi	Potential mycotoxins
Fusarium spp.	Deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol and other trichothecene compounds, zearalenone, moniliformin, fumonisins
Alternaria spp.	Alternariol, alternariol monomethyl ether, altuene, tenuazonic acid
Penicillium roqueforti	PR toxin, patulin, roquefortines, penicillic acid, mycophenolic acid
Aspergillus fumigatus	Fumitremorgens, verruculogen, fumigaclavines, gliotoxin
Aspergillus flavus	Aflatoxins, kojic acid, cyclopiazonic acid
Aspergillus parasiticus	Aflatoxins
Byssochlamys spp.	Patulin, byssochlamic acid
Paecilomyces varioti	Patulin, byssochlamic acid
Monascus ruber	Monacolins, citrinin

Due to insufficient cleaning of the silos, the silos are constantly present there and contamine the incoming product. However, it is at the time of actual harvesting that the storage moulds contaminate the grains. In contamination, soil, stalks, leaves do not play a primary role. However, the mowing method is important. Contamination with *Penicillium spp.* is 250 times higher in harvest with harvesters compared to manual harvest. The elevator of the harvesters and the inside of the storage tanks are the sources of contamination of the storage moulds. During the harvest, the grain's seed shells become contamined with conidiospores and mycelia. When the harvested grains are examined under the microscope, the ones that have not yet been seen as sport germs are not of low quality and if the fungus is noticed, they lose their qualities without entering the store. In subtropical climates such as Turkey, there are fewer problems due to harvest season and air temperature compared to cold-cool regions. In Europe and Canada, it is great importance that cereal and grainy products are dried up to a certain moisture content in special drying facilities. Figure 1 shows the contamination of the toxin in the production chain (3).

It was seen that when dried wheat was stored on condition that moisture content of grain is 13. 8-14. 3%,the wheat embrio was contaminated only wıth Eurotium halophilicum. This is not taken care of much because it develops slowly. In the case of moisture of grain is 14-15%, the kserophile characteristic primary invasive fungus occur. These are *Aspergillus restricus, Wallemia sebi, Eurotium amstelodamii, Eur. Chevalieri, Eur. herbarium* and *Eur. rubrum* cause the grains to be covered with yellow colour due to the cleistothecenes (cleistothecien, closed askocarps) they produce. If the moisture content is 15. 5-16% in storage grains, *Asp. Candidus, Asp. Achraceus* is

visible. Moisture content of around 17% causes the *Eurotium* genus to dominate *Aspergillus* with other species, but the production of kleistothecenes regresses. If the contents of the moisture are in the range of 17-19% *Asp. Flavus* begins to develop. In a 20% or greater moisture content product range, the *Asp. Flavus* is 50% weighted. In addition, such as *Asp. Niger, Asp. Fumigatus, Asp. Filamentous* fungi terreus also occur (3).

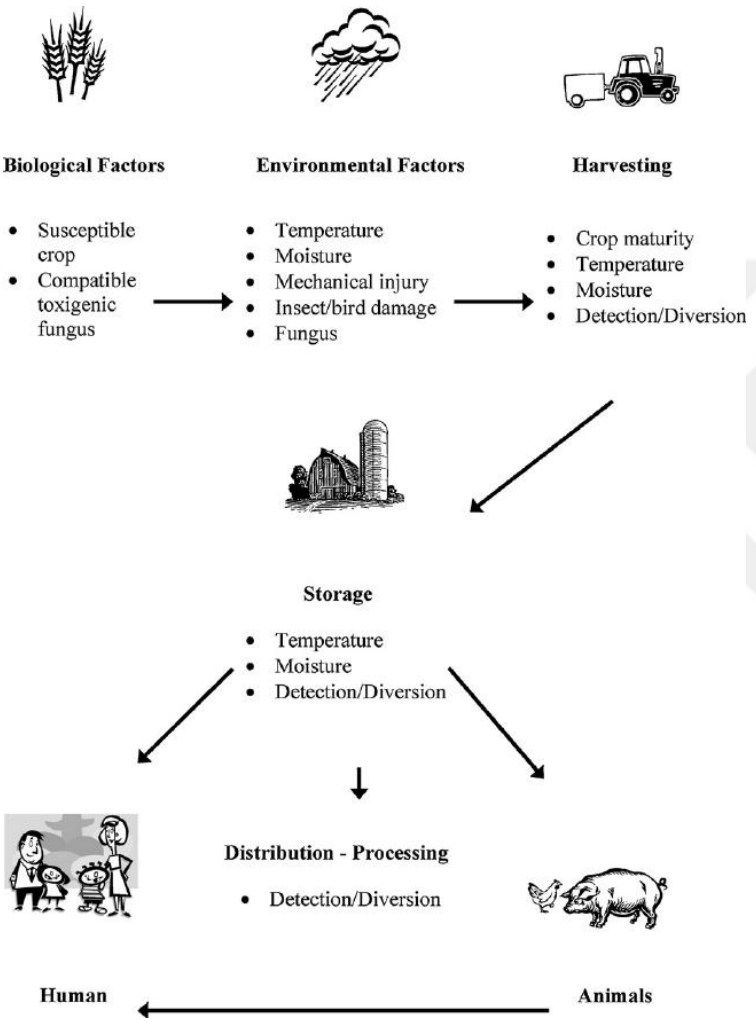

Figure 1: Mycotoxin contamination at production chain (5).

1.3 Classification of Mycotoxins, Interactions and Toxıcologıcal Investigations

1.3.1. Aflatoxins

Aflatoxins, are secondary metabolites of certain strains of A. flavus and A. parasiticus fungi. Aflatoxin B1 (AFB1) and AFB2 give blue fluorescence under ultraviolet light and AFG1 and AFG2 give green fluorescence. Being wıth similar structure, the major aflatoxins are AFB1, AFB2, AFG1 ve AFG2. Although these toxins are present in varying amounts in various nutrients and seeds, AFB1 is usually the most efficient. Figure 2 shows AFB1, AFB2, AFG1 and AFG2, which are the major aflatoxins. Table 2 shows the physical and chemical properties of aflatoxins (6).

AFM1 and AFM2 are the compounds found in the milk of cows fed with feeds containing AFB1 and AFB2, similar to the main molecule but with less biological activity.AFM1 is the major catabolites of AFB1 in animals and is usually excreted in milk and urine. (6)

Aflatoxins can be divided into two groups according to their chemical structure, difurokumarocyclopentanone and difurokumarolactone.AFB1, AFB2, AFB2a, AFM1, AFM2, AFM2a and alfatoxicol are in the difurokumarocyclopentanone group; there are AFG1, AFG2, AFG2a, AFGM1, AFGM2, AFGM2a and AFB3 in the difurokumarolactone group. Compared to the dihydrofuran group structurally containing a double bond and the functional groups attached to the coumarin group, the severity of the formed biological effect may vary. Demethylation of AFB1 is a toxic derivative of AFP1 and Hydroxylation of the carbon atom at the bridge site to the furan rings also results in the formation of AFM1, which has similar effects with AFB1 but is less carcinogenic (7).

Aflatoxins are common contaminants of corn, peanuts, walnuts, Brazilian peanuts, flaxseeds, other foods with high carbohydrate content, and even plants and spices. Starting from the time they are planted in the field, food can be contaminated at every stage of growth, harvest, transportation, poor storage conditions, conditions during production, and even shelf life of the product used as ready-to-eat food, in short, from planting to consumption (8).

It is called for aflatoxicosis the toxicity table formed with aflatoxins. Humans can be exposed directly to aflatoxins through products obtained from occupational exposures, or especially from animals fed with contaminant feed. (9).

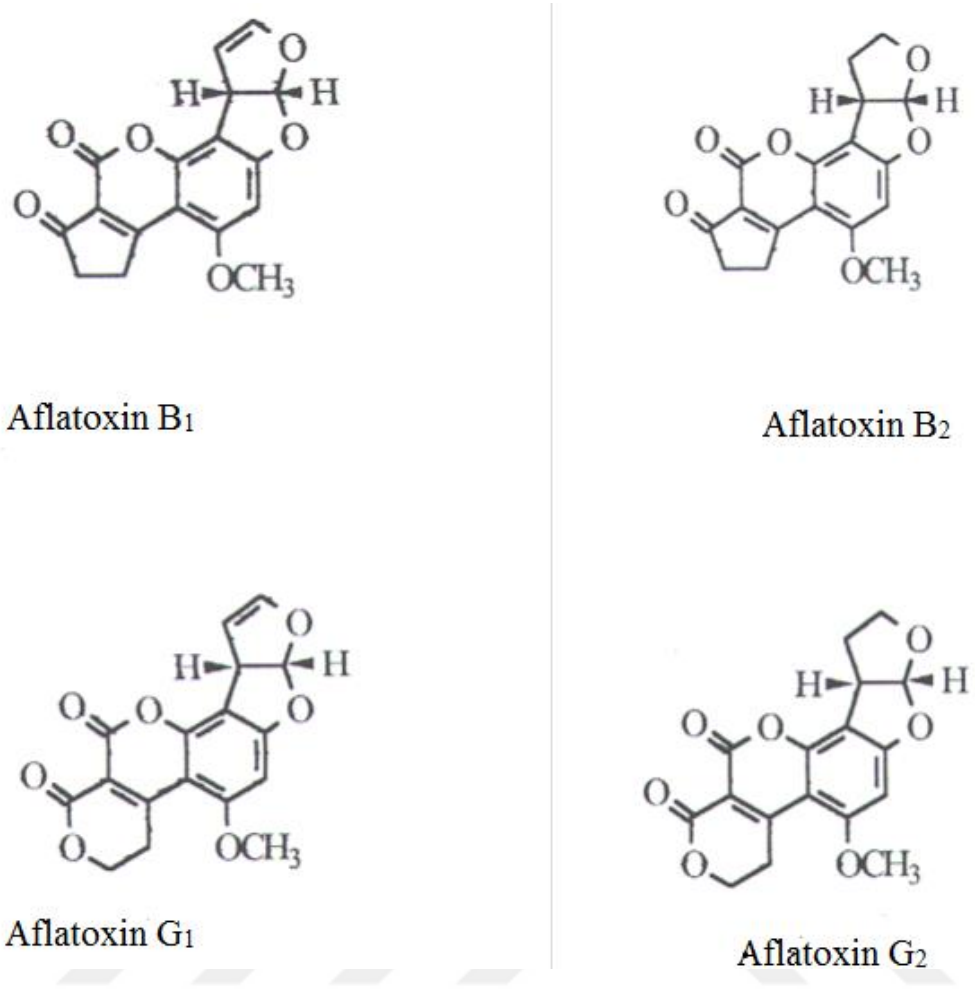

Figure 2: Some aflatoxin structures (7).

Table 2: Chemical and physical properties of Aflatoxins (7).

Aflatoxin	Molecular formula	Molecular weight
B_1	$C_{17}H_{12}O_6$	312
B_2	$C_{17}H_{14}O_6$	314
G_1	$C_{17}H_{12}O_7$	328
G_2	$C_{17}H_{14}O_7$	330
M_1	$C_{17}H_{12}O_7$	328
M_2	$C_{17}H_{14}O_7$	330
B_{2A}	$C_{17}H_{14}O_7$	330
G_{2A}	$C_{17}H_{14}O_8$	346

1.3.2. Contamination of Foods with Aflatoxins

The contamination of aflatoxin with, feed for farm animals and foodstuffs that are beneficial to human nutrition, is a frequent occurrence in various parts of the world. Therefore, these contaminations both affect the food safety and create a risk for public health as well as causing significant economic losses in the agricultural industry (10).

Aflatoxin is most commonly found in herbal products. Among peanuts, hazelnuts, pistachio nuts, almonds, pine nuts, various walnuts (Paraguay walnut, pecan walnut, coconut), peanuts and products are the most risky foods. At cereal group, wheat, corn, rye, barley, oats, rice may be contaminated with aflatoxin and their milling and bakery products are also at risk. Various flours, bran, semolina, corn flakes, spaghetti should be considered in this context. In legumes soy bean stands out, but they can be seen in beans, peas, cowpeas, lentils. Aflatoxin is often found in oil seeds cotton, sunflower, sesame and rapeseed seeds. Higher visibility in these seeds and other products with high oil content is explained by the high proportion of unbound water required for the growth of moulds. Depending on the raw material; Peanut paste, hazelnut paste, almond paste, marzipan (almond or apricot kernel paste), persipan (peach kernel paste), (Groundnut Toffees) prepared from chopped ground nuts or whole chopped hazelnuts have risk. Feedstuffs such as sorghum, millet, spice and various seeds (groundnut, soybean, cotton, sunflower seeds) are highly contaminated with aflatoxin. Especially red pepper, pepper, black pepper and dried fig fruit are the leading risky products in terms of aflatoxin (3).

Spice varieties were also investigated in terms of aflatoxin content. Red pepper (dust and flake) is the most risky product. In black pepper, aflatoxin is less common and the concentration is lower. Saffron, ginger, coriander, powdered or chopped coconut is a kind of spices found aflatoxin positive. In contrast, cinnamon, mustard, fennel grass, anise, cardamom, curry, Spanish red pepper (paprika) does not contain aflatoxin. However, the types of spices found to be negative reflect the results of a few scans. While no data were available for cumin, cumin and anise seeds in *Asp. Parasiticus*, cardamom in *Asp. Aflatoxin* was formed on these substrates when inoculated with flavus (3).

Corn peppers in black pepper (dark and light colour) are known to be of poor quality. In this country's black pepper *Asp. Flavus* and *Asp. Parasiticus* forms dominant flora. Isolated Asp. 20% and 100% of the flavus strains were able to produce aflatoxin

according to two different studies. No aflatoxin was detected in dark and light colored varieties of black pepper samples (n=23), In dark pepper corn pepper, 35 $\mu g.kg^{-1}$ in the dark colour and 22 $\mu g.kg^{-1}$ AFB1 in the light colour varieties were determined. In terms of aflatoxin formation, red pepper is a suitable substrate that can not be compared with black pepper. Analyzes made for control show that red pepper is considerably contaminant. Red pepper, which is called, Albanian pepper, red pepper from India, Ethiopia, Egypt and Turkey, is in a very bad condition for aflatoxin. 50% of powdered red pepper samples tested in Germany were aflatoxin positive and 18% contained 8.4 - 24 $\mu g.kg^{-1}$ AFB1.It has also been determined that 50% of the red pepper samples (n=2000) in the German market, mostly imported from Turkey, contain aflatoxin above the limit values. Aflatoxin contamination was determined as 66.7% in red pepper samples (n=60) from Kahramanmaras and Gaziantep in Turkey And OTA contamination were determined as 33.3%, while those exceeding the limit value were found as high as 46. 7% . (3).

It is not possible to produce aflatoxin with direct mold contamination in animal products. Aflatoxin is not as risky as meat and meat products, if not taken into account, raw fermented sausages, which are produced in homes and farms with the natural method and matured with natural mould flora and salted and dried ham. Artificially fresh meat and ham contaminated with, *Asp. Flavus* and *Asp*. *parasiticus* significant levels of aflatoxin have been formed in, provided that they are kept at 20 °C for 14 days. However, market surveys revealed that aflatoxin was not found in meat, salami and sausages. It can easily be said that there is no aflatoxin in meat that is processed properly, ripened and stored (3).

Milk, liver, kidney, eggs are contaminated with aflatoxin via "Carry over" .At critical aflatoxin concentrations of feed, toxins pass to organs and tissues with 0.01-0. 3% (3).

1.3.3. The Factors Affecting Aflatoxin Formation

Moisture and temperature are important parameters in the development of aflatoxins. Optimum conditions for the development of aflatoxigenic moulds are relative humidity of 24-35 °C and above 70%. In addition, aflatoxin production and

mycelial growth are controlled by temperature and water activity (aw).Other factors are temperature application, modified atmosphere packaging and preservative use. For this reason, air or sun-drying grain is a common contaminant in seeds such as cotton, peanuts and hazelnuts. Corn is also commonly exposed to mycotoxins in the growing, harvesting, storing, transporting and processing stages. If the drying and storage conditions are not good in corn infected with *A. flavus* before and after harvest, there is an increase in aflatoxin contamination. (11)

The temperature and water activity (aw) values required for mould development are not the same as those required for toxin formation and vary by species. For example, in the development of *A. flavus* and mycotoxin production, aw is 0,73 and 0,85, respectively. In this case, the moisture content is 8-12% and 17-19%. (11)

Minimum temperature values required for mould and toxin formation also vary. For the development of parasiticus, the optimum temperature range is 25-35 °C while the lowest temperature range is 6-8 °C. For *A. flavus*, the best growth interval is 19-35 °C while toxin is produced at 12-42 °C. (11).

Aflatoxin contamination can occur rapidly. In one study, the presence of aflatoxin was found in plants inoculated with *A. flavus* in amounts of 0. 3-2 ppb two days, 950-2800 ppb four days later and 3.600-4.500 ppb seven days later.

1.4. Detoxification and Decontaminations of Aflatoxins

The aflatoxin problem is not only a serious threat to human health but also of economic importance. It is stated that the economic losses have reached billions of dollars in the world. due to this reason. In 1985, FAO calculated that 25% of world food production was contaminating with mycotoxins. These economic losses caused the manufacturer's product loss, animal and milk losses, leading to the high costs of the operator and distributor, and finally the consumer being adversely affected by high prices and rising health costs. In addition, legislative costs, research and training costs in this regard also place an additional economic burden (12).

For all these reasons, the prevention of aflatoxin formation in food and feed is important. Prevention of aflatoxin formation should primarily prevent mould

contamination during the development, harvesting, storage, transportation, processing of the product and cropping of the raw materials in the field. It is very difficult to keep microbial contamination under control in the field. However, microbial contamination can be largely avoided with hygiene and sanitation precautions and conscious practices to be taken at harvest and subsequent stages. The second and even more important step in the prevention of aflatoxin formation is the prevention of the development of moulds / moulds in raw materials, intermediates and end products in various forms. This may be possible using good technology and conscious practices in production. The prevention of mycotoxin formation has great difficulties and often fails because moulds have a low developmental desire and accordingly, they can grow almost everywhere and conditions. In cases where aflatoxin contamination can not be prevented, numerous studies are conducted to remove aflatoxin from the product and to detoxify, Physical, chemical and biological methods are used. (13-14).

1.4.1 Physical Techniques for Aflatoxins Reduction

Physical separation methods are widely used to reduce aflatoxin levels with manual or electronic means of extraction. The best results were obtained in the peanut sector to reduce aflatoxin by sorting out variegated, distorted, and deformed grains. Products such as corn or cottonseed are not used frequently because of difficulties in applying this method. The products can contain significant levels of mycotoxins, even if they do not show a mould deterioration. Therefore, even if low aflatoxin levels are reached in the last flush from the beginning with the sorting, often the contamination can not be completely eliminated. Among the methods of physical decontamination, the effects of ionizing and non-ionizing radiation, solvent extractions, adsorption and microwave heat treatment on aflatoxin are also examined (15).

1.4.2. Chemical Techniques for Aflatoxin Reduction

Food additives and chemical adsorbents are also considered as potential decontamination methods. We have examined a large number of food additives from this direction and have observed that some of them reduce the level of aflatoxin added to food. On the other hand, successful results have been obtained in the removal of patulin and ochratoxin A by activated carbon. Some non-digestible adsorbent materials

are used commercially in forages. There are also studies in which physical and chemical detoxification methods are used together. Ammonification in chemical contamination procedures has been legally accepted in some countries and is widely used in forage raw materials in these countries (15).

1.4.3. Biological Techniques for Aflatoxin Reduction

The different methods investigated for the removal of aflatoxin from the product, although successful at certain degrees, they have some disadvantages these are, not achieving adequate levels of detoxification, causing loss of food items, high cost requirements. For Many researchers working on this field; the best solution for decontamination is biological detoxification and they think that it will prevent the use of hazardous chemicals and cause significant loss of nutritional value and renewability in food / feed (15).

One of the most studied biological methods is the removal of mycotoxins by fermentation. In two different studies, the amount of toxin was monitored during the ethanol treatment from corn contaminated with *Zeralenon* and *Fumonisin*; While toxin was found in ethanol produced in both, it was determined that the toxin remained in other fractions (15).

In a study of the effect of alcohol fermentation on tricothecenes, it has also been shown that tricothecenes are transformed into their derivatives. In one study, deoxynivalenol (DON, *vomitoxin*) and *fumonisin* were found to be stable in ethanol fermentation. Another study showed that during the alcohol fermentation in beer production, three *Saccaromyces cerevisiae* strains reduced the *ochratoxin* in the malt, *fumonis* B_1 and *B.* It has also been reported that grape juice is also tricothecene - like during alcohol fermentation. Number of studies have examined the effect of microorganisms on various mycotoxins, The microbial mixture of *ochratoxin A* was isolated from the soil by three bacteria isolated from the soil, diacetoxysirpenol and a derivative of which was isolated from the soil by yeast cultures of zeralenon. In recent years it has been reported that positive results have also been obtained from studies conducted on the removal of aflatoxin by lactic acid bacteria (15).

1.5. Methods Used in Aflatoxin Analysis

In general terms, the test procedures and analyzes for mycotoxins are usually made by way of sampling. Variations of the assay results obtained vary. Mycotoxin levels are not 100% accurate due to the different results of the analysis, depending on whether the sampling plans are done correctly. In addition, some good results can be rejected, or bad results can be regarded as accurate. HPLC and TLC methods are frequently used with regard to reduce these analysis errors. Capillary electrophoresis methods have also begun to be used depending on the technological developments in recent years. The feasibility of electrochemical flow injection methods is also being investigated. Examination of possible interference effects on the determinations of protein and lipid present in the medium is also a consideration. These effects can be removed by changing the carrier electrolytes and adjusting the flow rates. The results obtained in an HPLC study using an electrochemical detector are compared with TLC, indicating that both methods are in harmony with each other (16).

Due to its superiority over other analytical methods, the fluorescence detector HPLC method has become a widely used and acceptable method in AFL determinations. Although both normal and reversed phase chromatographic techniques can be used, reverse-phase HPLC methods are frequently used. In an HPLC method developed for the blend of aflatoxin B1 and aflatoxin B1-oxime, analyzes were performed using UV and fluorescence detectors (16).

The HPTLC method was also used for aflatoxin determinations in corn. Researchers emphasize that this method is faster and more economical than other HPLC methods. A method called overpressured-layer chromatography (OPLC), which is formed by combining the advantages of HPLC and HPTLC methods, appears to have begun to be used in recent years. In this method, the components are first densitometrically separated and collected and then automatically sent to HPLC columns (16).

In an study to increase the fluorescence properties of aflatoxins, the changes in signals have been investigated by adding cyclodextrins to mobile phase. Especially for Aflatoxin B1 and Aflatoxin G1, these joints have significantly increased signals. Addition of β-cyclodextrin resulted in an increase of 11. 6 fold for Aflatoxin B1, 10. 2 fold for Aflatoxin G1, 27. 5 fold for Aflatoxin B1 and 13. 9 fold for Aflatoxin G1 with

addition of succinyl-β-cyclodextrin. It has been reported that a significant increase in signaling causes the limits of detection to fall to 0. 0005 μg / kg (16).

1.6 Taking Aflatoxins to Body and Their Effects

Mycotoxins reach people at the level of residues via products, contaminated food, obtained from animals fed with contaminant foods. Approximately 25% of the nutrients are in underdeveloped or developing countries appeared to be contaminating with mycotoxins and metabolites (17). Mycotoxins are usually taken in the body by consumption of contaminating food, but other ways of exposure to contact with the skin, such as inhalation of toxic spores. Daily amounts of aflatoxin intake in different parts of the world vary between 0-30,000 ng kg^{-1} (18).

Humans can be exposed to toxins in low amounts by diet. Exposure to low doses of aflatoxin in the long term can lead to very dangerous results. Some health factors such as aflatoxin exposure status, age of the person, nutritional status, hepatitis B infection affect the toxicity of aflatoxins (19).

The acute and chronic mycotoxicosis that aflatoxins form in humans and animals is called aflatoxicose. It is also stated that aflatoxins may cause cirrhosis, hepatitis, chronic gastritis, Reye's syndrome and kidney diseases. In addition to, it is reported that aflatoxins are associated with Kwashiorkor's disease in children. Aflatoxin is found in human cord blood, and the it can implicate to developing fetus. In addition, mycotoxins can cause loss of yield in animals, decrease in weight gain, immunosuppression and cancer. Poisoning can occur in animals fed with contaminated feed (19).

Aflatoxins are lined up AFB1>AFG1>AFB2>AFG2c according to their toxic effect. Among these toxins, AFB1 is the most toxic and most prevalent in terms of human health. Aflatoxins have acute and chronic toxicity, and most of them have carcinogenic, mutagenic and teratogenic effects. AFB1 Group 1 carcinogen, AFM1 Group 2 carcinogen have been reported by the International Agency for Research on Cancer (IARC). The main target organs for the toxic and carcinogenic effects of AFB1 are liver and kidney. Hepatotoxic, hepatocarcinogenic and teratogenic effects of aflatoxin were shown in different animal species.AFB1 can induce malignant tumor

formation in some animals such as rat, mouse, monkey, marmoset, duck, guppy, salmon, trout, and chrysanthemum (11).

There is no effective treatment method for mycotoxin poisoning. It is also unlikely that contaminating foods are free from mycotoxins. For this reason, it is important to avoid consumption of contaminant foods in terms of human health and take effective measures against food contamination (11).

1.7 Aflatoxinity in Food and Legal Regulations

Aflatoxins are commonly found in raw peas, hazelnuts, cocoa, coffee, corn, rice, wheat and other cereal products and dried fruits from raw food consumed in the world. It can also be widely found in many foods, including spices such as, red pepper and red pepper products, dairy products. Longer consumption of aflatoxin-containing foods in high quantities can be a problem for public health and cause economic loss in the country since it can affect exports negatively. Therefore, the traceability of the development of mycotoxins from production to consumption is important (11).

There are legal restrictions on aflatoxin contamination in greenhouses in Turkey as in many other countries. According to the Turkish Food Codex on the maximum limits of contaminants in foodstuffs 2008/26 prepared in the context of European Union harmonization process, for many foodstuff, AFB1, total aflatoxin-TAF (B1, B2, G1 and G2) and AFM1-related limitations have been reported (20).

In many of the Asian countries rice is a basic food for aflatoxin density and contamination. In addition, aflatoxin has been reported in barley and corn based foods. It is known that aflatoxin is especially high in seeds. If the moisture is 8% and the ambient temperature is above 25 ° C, the aflatoxin-forming moulds can occur (21) .

Turkish Food Codex Communiqué, the peanuts are subject to physical processes such as sorting, sorting, etc. before being served directly to the consumer or before being used as a food component, maximum AFB1 in peanuts and total aflatoxin amounts of 8 and 15 μg kg^{-1}, respectively. In the same Communiqué, the maximum amounts of AFB1 and total aflatoxin of cereals (including buckwheat - *Fagopyrum sp*.) and processed foods produced therefrom were reported as 2 and 4 μg/kg^{-1}, respectively.

In addition to, the maximum total amount of aflatoxin was 10. 0 μg kg^{-1} for hard shell nuts such as pistachio nuts, peanuts, oilseeds, dried fruits and processed foods from these. In Table 3, Permitted limits for mycotoxins in various species are given (11).

Milk and dairy products are a good source of nutrients for people, especially for children, such as animal protein, calcium, vitamins and essential fatty acids. But milk and dairy products are also potential sources of aflatoxins. AFM1 can be detected in 12-24 hours from the receipt of AFB1.In milk-based products, the AFM1 product is not affected by the processing steps (22). Also, AFM1 is resistant to the pasteurization process in milk. There are legal restrictions on milk and dairy products, according to this, it has been stated that the maximum amount of AFM1 in milk used in the production of raw milk, heat-treated milk and milk-based products should be 0.05 μg kg-1 (11).

The maximum amount of AFB1 for infants and small children is specified as 0. 10 μg kg^{-1} . Also, the maximum AFM1 in baby foods (including infant milk and continental milks) should be 0. 025 μg kg^{-1} and the maximum AFB1 and AFM1 in diets for special medical purposes for infants are reported as 0,10 μg kg^{-1} and 0,025 μg kg^{-1}, respectively. The maximum values for AFB1, aflatoxin and AFM1 were reported as 5. 0, 10. 0 and 0. 5 μg kg^{-1}, respectively, for possible risky foods with aflatoxin. Virdis et al. investigated AFM1 contamination in cheese made from goat's milk and goat's milk in Italy. 4 (9,8%) of the 41 cheese samples were reported AFM1 at 79,5-389 ng kg^{-1} levels (20).

However, spices such as pepper, turmeric, black pepper, coriander and dry ginger can be contaminated before harvest, after harvest, during storage and transportation. In the Turkish Food Codex Communiqué, the maximum amounts of AFB1 and total aflatoxin in products such as Red pepper (*Capsicum spp*.), Black pepper (*Piper spp*.), Jellyfish (*Myristica fragrans*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) were 5. 0 and 10. 0 ìg kg^{-1}, respectively. One of the most consumed foods is red pepper and products. Redpepper is a very popular spice that is common in the world. Turkey is one of the countries that produce high amounts of redpepper like India, Mexico, America, Spain. Red powdered peppers are a kind of spices that are used frequently in Turkey for the color, flavour and flavour of the dishes. Redpepper; is one of the products sensitive to the formation of aflatoxin due to the conditions during

production, harvesting, drying and subsequent processing. The redpepper is the type of spice containing the highest level of aflatoxin. In the contamination of these peppers with mycotoxins, many factors such as climatic climate, crop genotype and soil type are influential (11).

Countries with the highest number of pepper producers in the world are located in tropical regions. Therefore, there are suitable climatic conditions for the development of mycotoxin-producing moulds in redpepper and its products. However, peppers are generally dried outdoors under insufficient hygienic conditions. Redpeppers, like many other prducts, can be contaminated with aflatoxins before harvest, after harvest, during storage, and during transport. In the 2008/26 Turkish Food Codex Communiquı on the maximum limits of contaminants in foodstuffs, the maximum amount of AFB1 and total aflatoxin for all powdery red repper is 5 μg kg^{-1} and 10 μg kg^{-1} respectively. Turkish Food Codex Communiqué was prepared under the harmonization process of the European Union, taking into account the Commission regulation on the determination of the maximum quantities of certain contaminants in foodstuffs 1881/2006 / EC. This directive was revised in 2010 with the directive 165/2010 (11).

Table 3: Permitted limits for mycotoxins in various species (23).

Mycotoxin	Feed stuff(s)	Limit (ppb)	Country / Authority
Aflatoxin B1	Maize	5	Turkey, Russia, Egypt.
	Maize	10	China, Korea, Japan
	Animal feed	10	Egypt
	Animal feed	50	Turkey
	All cereals except rice and maize	2	EU
	Unprocessed maize and rice	5	EU
	Animal feed ingredients	20	EU
	Feed stuffs for immature animal	20	FDA
Aflatoxin B1& G1	Maize	30	Brazil
Aflatoxin M1	Milk	0.5	U.S.A, Russia, Egypt
		0.05	Turkey
	Milk and milk products	0.05	EU
	Milk	0.5	FDA
Deoxynivalenol	Unprocessed cereals other than wheat, oats and maize	1250	EU
	Unprocessed wheat and oats, maize	1750	EU
	Cereal products	500	EU
	Cereals and cereal products for feed	8000	EU
	Maize by-products for feed	12000	EU
	Animal feed	100	FDA
Fumonisin B1, B2	Animal feeds except Equines	50	EU
	Animal feeds for Equines	5	EU
Fumonisin B1,	Animal feeds except Equines	30	FDA
B2, B3	Animal feeds for Equines	5	FDA
Fumonisins	Unprocessed maize	2000	EU
	Maize products for human	1000	EU
Ochratoxin A	Unprocessed cereals	5	EU
	Cereals and cereal products for feed	250	EU
	Cereal products for food	3	EU
T-2 and HT-2	All cereals grains	100	EU
Total aflatoxin	Animal feed ingredients	50	EU
	Animal feed	20	Canada, Egypt, Iran
		50	Brazil
	Maize	10	Turkey, Egypt
		30	India
	Cereals feedstuffs	200	Mexico
	Feedstuff (ingredient)s	20	Japan, U.S.A, Korea
	All cereals except rice and maize	4	EU
	Maize and rice	10	EU
	Feed stuffs for mature animal	100	FDA
Zearalenone	Unprocessed cereals other than maize	100	EU

1.8 General Information

1.8.1 Places Where Used for Spices

Spices are used in different types of food in kitchens, such as roasting or grinding in different shapes or mixes. The black pepper (*Piper nigrum*) from the family Piperaceae is collected from the tree on its shell without full ripening. It is used to give flavour and taste to meat, sauces and salads thanks to the flavour of the burning. Redpepper (*Capsicum annuum*) is used in salads, soups, meat dishes and some sauces.

Mint and thyme belong to the Labiatae family with aromatic sensory properties. Mint (*Mentha piperita*) is added to various herbal teas as it is put in various salads, vegetables, some sauces and some cold drinks. Thyme (*Thymus vulgaris*) is added to soups, meats, fish and vegetables. Cumin (*Cuminum cyminum*) which is grown in Konya and Ankara region in our country, which is the main homeland of India, is used in meat dishes, soups, sauces and fish. Cinnamon (*Cinnamomum zeylanicum*), which is the motherland of Southeast Asia, is added to beverages such as tea, salep, and boza, which are used in some meats and especially in desserts. Donut (*Nigella sativa*) is planted on cakes and breads without bake, and is added as a spice to cakes and biscuits. Sesame is used for (*Sesamum indicum*), bagels and similar paste foods. In our country, sumac (*Rhus coriaria*), especially grown in Southeastern Anatolia Region, is used in salads and olive oil. Carnations (*Syzygium aromaticum*), soups, meat dishes, cakes, fruit salads. Ginger (*Zingiber officinale*) is added to soups, dolmalara, grilled meats, pilaf, white cheese, various vegetables, pastries and cakes. Pimento (*Pimenta offcinalis*) is involved in various meat dishes, some desserts such as baklava and some drinks. The motherland of coriander (*coriandrum satiuum*) is India and Fas and it is used in all kinds of onion dishes, breads and some meats. Basil (*Ocimum basilicum*) which is grown in the Mediterranean region in our country and comes from the Labiatae family, which includes mint and thyme, is used in soup, fish and green salads. Fennel (*Foeniculum vulgare*) is used in soups, sauces, meats, fish grilles and canned foods (24).

1.8.2. Definitions

1.8.2.1. Spice

Spice, as the root word, is the plural form of fragrant and beautiful spring in Persian. In this study, the spice was evaluated as singular. It is described as one of the oldest languages of Iran, Avesta (or Zend language), as a generic name given to plants that open after rain in fragrant flowers and garbage used as vanhers. (24) .

1.8.2.2. History

The history and use of spices extends to the beginning of mankind. The use of spices in history is considered to be the first far east. Spices are the most important commodities traded on the earth. Spices have maintained their importance throughout history. So the way the spices are moved is called Spice Road. Over the centuries, wars have been made to capture the spice trade and this special way, and the American continent has been discovered while trying to change this path (24).

The use of spices dates back 4,000 years before Jesus, which is called the Neolithic age, not being fully open. These people, who are hunting and fishing, begun to cultivate, and thought the accumulation of wild scented plants found in the surroundings, after the of the nomadic settled. (24).

China and India is the motherhome of many spices. In India there were also other aromatic plants such as black pepper, cinnamon, cloves, small coconuts, cumin, and so on. Because of the natural richness of plants and spices, primitive drug production has developed in India. For example, in terms of health, basil was used for stomach and kidney disorders. Some spices were considered sacred. The coriander, which is devoted to God, was also a sacred spice and took part in magic rituals. When Marko Polo traveled to India, he said "they had all kinds of scented plants that needed health, other than black pepper, carnations, and coconut."Turmeric, which has a great importance in Indian medicine; cold, cough, liver disorders, rheumatism, sinusitis and anorexia. It is also used in the treatment of skin diseases. The first spice trade began in the Middle Ages with Chinese, they sold spices to Arabs passing through India. The Arabs also sold to Europe through Anatolia. This trade route extending from Chinese to Anatolia is called as silk road. The spice trade, which has been in the hands of the Arabs for a long time, has passed Portuguese and then the Dutch. In the thirteenth century, with the discovery of Asia by Marco Polo, Venice became a trading center for spices in Europe. In antiquity, a significant portion of world trade was spices. Spices were taken to the countries, from which were miles away from the countries where they were produced, and many livelihoods of peasants, merchants and workers were provided in this way. One of the earliest known civilizations, Herodotus, indicates that fragrant plants grew in these lands about the civilization of Assyria-Babylonia. Black pepper, ginger, cinnamon and turmeric coming from India and Chinese are added to products of the Babel gardens. Milat 2000 years ago, tablets belonging to Hittite civilization living in Central Anatolia were found, we can see spice names such as laurel, mustard seed,

saffron, onion, garlic, sesame seeds on these tablets. It is thought that the tablets located in Hattusas, the capital city of the Hittite civilization, are of the same quality as the Mesopotamian Civilization in terms of medicine. Although the meaning of all the plants mentioned in the tablets is unknown, bay, seeds of mustard, saffron, garlic, onion, sesame were ascertained (24).

Long before Christianity, ancient Chinese writings prove that spices are used in medicine. In this article, which is considered as a medicine work, it is understood that 12 spices are named and they are all used together as medicine and spice. In addition to the spice, fragrant plants such as anise, dill, fennel and especially chinchilla were grown in Mongolia and Tatarstan. These scented plants chewed and used as scents in religious ceremonies (24).

The spice was sacred in Egypt, and there was also a great importance in medicine. Egyptian medical experts, who first revealed the germ and bactericidal properties of spice and fragrant plants, treated the diseases with scented oils. Egyptians were the first to find a method of extracting essences from the crusts of the fragrant woods such as cedar and juniper and the bahamas (24).

An interesting aspect of the ancient Egyptian medicine is the mummification process. The Egyptians were the first to benefit from the antiseptic effect of cinnamon, carnations and small coconut. According to the knowledge given by Herodotus, the internal organs were cleaned with a special wine, then added with spices mixed with black pepper, cinnamon and ginger (24).

It is known in ancient civilizations that many spices are known, produced and used for various purposes. Historical records in Mesopotamia refer to spices such as mustard, thyme and saffron. At the beginning of the 15th century, the use of spices in food products became important, while in the early ages spices were used as crude. This situation lasted until the 19th century. In parallel with the developments in the science of chemistry in the 19th century, effective methods of spices were started to be determined and derivatives were obtained by various methods (24).

The use of ginger in medieval Europe was as common and as expensive as black pepper. Saffron coming from Iran and Phrygian and kashmir for medicine and paint, was known and used by Romans (24).

1.8.2. 3. Classification

Spices; According to the organ structures of the plant;

1) Leaves and vegetables (redpepper, greenpepper, mint, duck, thyme etc.)

2) Root (cowberry, salep)

3) Fruit and seeds (fennel, blackcurrant, coriander, mustard, vanilla, black pepper, coconut, caraway, pimento, mahlep, sumac)

4) Flowers and branches (cinnamon, cloves, linden) are classified as (25).

Spices can also be classified according to the family to which the plant belongs, according to their sensory properties (such as aromatic, burning, coloured, phenolic etc.) or according to the composition of the essential oil.

1.8.2.4. Chemıcal Composıtıon of Spices

Especially essential compounds that provide the characteristic of spices and non-essential flavors are colour substances. They contain different chemical compounds in the spices such as in the products and in the vegetables. These chemical compounds are (24):

1) Water: Spice water content is low unless it is consumed fresh. Water content after drying is between 5-12%.The water content should not exceed 14% to prevent microbiological degradation during storage. The water rate with the cause of crumbling should not be less than 5%.

2) Carbohydrates: In the spices, glucose, fructose, sucrose, maltose and raffinose are the most common. The amount of starch varies according to type of spice. Generally, fruit-seed spices are starchy.

3) Nitrogenous substances: Proteins are not an important contribution to the flavour of spices, but they give some sensory properties to the roasted products. Other non-protein nitrogen compounds have an important effect on the taste, smell and colour of spices.

4) Lipids: Lipids such as fixed oils, phospholipids, sterols and candles are found especially in the spices of fruits and seeds. Constant oils that do not add too much raw aroma, roasting and autoxidation form sweet and odorous compounds.

5) Glycosides: It is influential on the characteristics such as taste, smell and color of spices.

6) Alcoloids: Most of the alkaloids found in the spices are bitter, tasty and colorless, and some are strong smelling.

7) Tanens: Some spices from the family such as *Leguminosae, Anaca dioceae, Myrtaceae, Polygonaceae* and *Rosaceae* are rich in grain. Tannins, which are compounds in the non-nitrogen polyphenolic structure, provide the sour taste of spices in addition to their antimicrobial effects. It also has effects on colour.

8) Organic Acids: Malic, citric, tartaric and succinic acid are the most common organic acids. Sourness, which is the characteristic feature of acids, is not seen in spices. In addition to organic acids, spices such as rosemary, sage and dill include phenolic acids and diterpenic acids.

9) Vitamins: Many spices have great nutritional qualities. Especially freshly consumed leaf spices contain vitamin C.

10) Enzymes: The aroma formation with the enzyme effect in spices is very common in the family of *Liliaceae* and *Cruciferae*. Enzymes should not be too much in spices.

11) Colour Compounds: There are many compounds such as chlorophylls, which give different colors to spices, antresensitizers, flavonasides, quaternonoids and tannins, while leaf spices used fresh or stored frozen contain chlorophyll.

12) Minerals: Spices contain 3-10% of ash on average. This value is more in leaf spices.

13) Antimicrobials: Many of the *Liliaceae* and *Cruciferae* families have various compounds that have antimicrobial activity in many plants. In particular, essential oils such as rosemary, thyme, mustard, clove and cinnamon have a significant antimicrobial effect.

14) Sulfur Compounds: In Allium, some Cruciferae and Umbelliferae plants contain sulfuric substances. Certain enzymes influence these tasteless and odorless substances, and the sulfur compounds come into the form and give aroma.

15) Resins: Mixed materials composed of many monomers and polymer compounds. It is usually found with essential oils, gum or both. Resins are antimicrobial effective, scented and stabilizing substances.

16) Essential Oils: Non-essential compounds give taste to spices, the fragrance comes from essential oils. Essential oils have an important role in imparting spices. Almost all of the spices contain essential oil.

In black pepper, rich oils rich in caryophilia create sweet floral flavor. The major component of fresh pepper is translinalol oxide and terpineol. Known as Gingerol , and give its fragrance and flavour to material, is in ginger's essential oil. Cinnamon takes its bitter taste again in cinnamaldehyde and cinnamyl acetone in its essential oil. The cuminaldehyde (4-isopropylbenzaldehyde) material in the cinnamon makes up a significant portion of the acute harshness. Coriander's special flavor is essential oils that aliphatic aldehydes are predominant (24).

1.8.3. Effects of Spices Health

Depending on the ingredients contained in the spices, various health effects are available. Animal and human studies on spices show that spices cause positive or negative changes in metabolism. Spices in all steps from production to consumption are at various risk of contamination. The negative effects of food contaminants contained in spices on human health are important for public health (24).

1.8.4. Adverse Conditions Caused by Spices

The content of spices, their properties such as soil and climate, their physical and chemical properties, product formulation, preparation method, storage, packaging, or presentation to the market, distribution pattern, shelf life, etc. are all important in terms of human health (24).

1.8.4.1. Microbiological Pollution

There is little information that plants and spices are effective in food diseases however there is a potential danger when is added food prepared before cooking or before cooking (24).

The plants from which the spices are obtained become in contact with these microorganisms as they are in contact with soil and water which are sources of many bacteria and fungi. Studies show that the spice is contaminated with moulds, aerobes and anaerobic spores with bacteria and pathogens. Especially growing in hot and humid areas and harvesting by complying with hygiene regulations increase the risk of contamination. Number of moulds and yeast is an important quality criter for foodstuffs especially those that are exposed to the open air prior to the packaging process for production technology requirement that are only grinded and packaged without any treatment or that are only processed such as washing, cooling and freezing (24).

1.8.5. Biology of Black Pepper

Latince Name: *Piper nigrum* L. Familya: *Piperaceae*

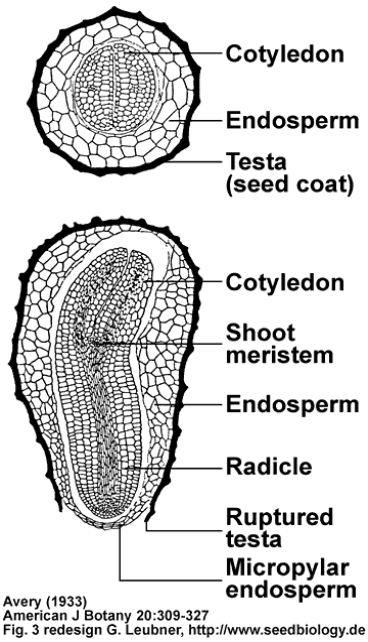

Figure 3: Black pepper section (25).

Plant: Indian origin, a perennial plant. It has a climbing and surronder structure like a vine, has thick leaves, 6-7 m in length and oval-shaped large leaves. There are small-white flowers in bunch form and there are about 20-30 fruits per bunch (25).

Black pepper grows in warm, humid climates such as areas near the equator in areas that receive 1500 mm or more rainfall. Lowering the temperature below 10 ° C is not good for plant growth. Reproduction of the plant is done vegetatively. The section of black pepper seed is shown in figure 4 (25).

The traditional large producers of black pepper are India, Indonesia, Brazil and Malaysia. The last participant in black pepper production is Vietnam and is the country with the largest blackpepper exporter. Unlike India, producer countries do not have a significant internal market. Sri Lanka is a producer of high-pepper extracts of piperine, the component responsible for the burning. It is also among the other countries producing blackpepper in Cambodia, Madagascar, Singapore, Sri Lanka, Thailand, Burmese and Comoros (25) .

1.8.6. Physical Properties of Blackpepper

Green fruits collected without full ripening will have a visible appearance in varying tones as from gray to dark brown and black when dried under the sun or fire. Black pepper grains are 4-6 mm in diameter, single seeded, wrinkled at varying ratios, whitish in their interior. Fruit is offered in the market under the name of black pepper if it is form of grain, and if it is grinded its name is powder black pepper. Grain blackpepper is the strongest taste and smell among the pepper varieties (25).

Near maturity or maturation, yellow-brown color ranging from red-brown to renge is removed by fermentation in water for a few days, the outer husk is removed and the sun dried and white pepper is obtained. Green pepper is the collection of immature green colored fruits and soaking them in salt water. The result is the result obtained (25).

1.8.7. Chemical Properties of Blackpepper

For every 100 g of black pepper consumed as a spice: 255 kcal energy, 10.5 g water, 11 g protein, 3.3 g oil, 64.8 g carbohydrate, 13.1 g fiber, 4.3 g ash,437 mg of

Ca, 29 mg of Fe, 194 mg of Mg, 173 mg of P, 1255 mg of K, 44 mg of Na, 1 mg of Zn mineral substances and 1 mg of niacin, 190 IUA of vitamins (25) .

Total ash in black pepper; It should be at most 7% ,Asitte insoluble ash; At most 1.5%, water; Up to 12%, crude cellulose, up to 12.5%, essential oil; At least 1.5% non-volatile methylene chloride extract; At least 7.5% and starch; Should be at least 30% (25).

1.8.7.1. Essentıal Components

Especially volatile oils make a big contribution to black pepper flavour and pepperberry contains 25% essential oil. The colour of the volatile oil obtained by steam distillation from black pepper granules varies from clear to pale green color, burning, spring and fresh flavour. (25).

1.8.7.2 Monoterpene Hydrocarbones and Oxygen Components

Studies on black pepper have identified 15 different monoterpene hydrocarbon compounds, these are kamphor, 3-karen, p-cymene, limonene, myrsene, cis-osmene, fellandren, -fellandren, and -pinenes, sabinen, and -terpinenes, terpinolen. Approximately 43 oxygenated monoterpene components were found and identified in black pepper essential oil These include: borneol, camphor, carvacrol, cis-carveol, trans-carveol, carvone, carvacenacetone, 1,8-cineol, krypton, P-cymene-8-ol, p-cymene-8-methyl ether, dihydrocarbone, dihydrocarbone, linalool, cis-2-mentadien- 7 -pentadien-6-ol, 1 -p-mentadien-4-ol, 1,8 ,1,8 -p-mentadien-4ol, cis-p-menten-1-ol, mirtenal, myrtenol, methyl carvacrol, trans-pinocarveol, pinocamphon, cis-sabinene hydrate, trans- 4-ol,1-terpinen-5-ol, terpeneol, 1,1,4 trimethylcyclohepta-2, 4-dien-6-ol, fellandral, piperitone, citronellal, nerol, geraniol, isopinocamfon, methyl citronellate, methyl geranate,Terpenolene epoxide and trans-limonene epoxide. Monoterpene hydrocarbons found in black pepper essential oil; -pinen, -pinen, sabinen ve limonen (25).

1.8.7.3. Sescihterpene Hydrocarbones and Oxygen Components

In black pepper essential oil there are also oxygenated terpene hydrocarbons. At the beginning of these - the caryophyll comes and the basic sesquiterpene

hydrocarbons found in black pepper oil. The main oxygen components are: Caryophyllene, -cis-bergamotene, -transbergamotene, -bisabolene, -and -feminine, calamen, -cophene, -and -cabenen, ar-kurkumen, - and -elemenler,-farnesen, -guain, - and -humulenler, isocarofiller, -urolen, -santalen, -and -selinenler, leden, seskisabinen and zingiberene (25).

Approximately 20 oxygenated sesquiterpenes are described in black pepper oil. These include: 5,10-cadinene-4-ol, caryofilamol-3, 7-dien-4-ol, caryofilam- 2,7-dien-4-ol, -caryophyllin alcohol, caryophyllene ketone, Cis-nerolidol, 4,10,10-trimethyl-7-methylenebicyclo- (6.2.0) decane-4-carboxaldehyde, cubenol, epi-cubenol, viridiflorol, and -bisabolol, cubebol,idiflorol, - and -bisabololIar, cubebol, elemol and -desmo (25).

Studies conducted by different researchers have shown that the chemical structure of black pepper is very variable. This varies depending on whether the plant is a culture plant or not, climate change, fruit maturity and the method of obtaining essential oil (25).

1.8.7.4. Aromatic Components in Blackpepper

Aromatic compounds identified in black pepper oil; Eugenol, methyl eugenol, myristidine, safrole, benzaldehyde, trans-anethole, piperonal, m-methyl acetylbenzene, p-methyl acetylbenzene, n-butyrophenone, benzoic acid, phenyl acetic acid, cinnamic acid and piperonic acid..Other components are;Methyl heptanoic, methyl octanoate, 2-undecanone, n-nonane, n-tridecane, n-nonadecane, and piperidine (25).

1.8.7.5. Non-essentıal Components

The basic flavour that gives flavor to black pepper is piper. Piperine is a yellow crystalline substance with a melting point of 128-130C. Piperine, a chemical formula C17H19O3N, is a weak base, which is then called piperidine, which gives essential property C5H11N1, which is hydrolyzed with aqueous alkali or nitric acid. Acidic product of hydrolysis; Piperine acid is shown as 5- (3,4-methylenedioxyphenyl) -2,4-pentadienoic acid (25).

2.MATERIAL AND METHODS

2.1 Material

Unpackaged black pepper samples obtain from certain herbalists in İstanbul, Eminönü (Spice Bazaar). For the 20 samples 20 different herbalists were observed and samples coming from the same storages were not bought. Each samples prepared in 100 gr packages to be examined in the labratory, in a position that they will not be contaminated.

Packaged black pepper samples obtain from certain herbalists, market and supermarket in İstanbul, Florya. Standart 16 samples prepared 100gr packages to be examined in the labratory, in a position that they will not be contaminated.

2.2 Methods

2.2.1. Mold Isolation at Blackpepper Samples

Plates containing Peptone Dextrose Agar used for isolation were left in the laboratory for 7 days at room temperature (22-26 ° C). During this time the ureters have been checked. After then each fungus colony that was breeding was taken by passage through the potato dextrose agar (PDA), Malt Extract Agar (MEA) and Czapek's Agar (CZ) broth. These plates were also incubated for 7-10 days at room temperature (22-26 ° C). At the end of incubation, pure cultures of microfungi were obtained. At this time, the surface and reverse appearance of colony and diameters are noted, the presence of exudation and pigmentation is examined (26).

2.2.1.1 Nutrient Environments and Preparations Used for Microfungi

2.2.1.1.A. Peptone Dextrose Agar

Dextrose 2. 0 g

Pepton 5. 0 g

KH2 PO4 (Potassium dihydrogen phosphate) 1. 0 g

MgSO4.7 H2O (Magnesium sulfate) 0. 5 g

Agar 15. 0 g

Distilled water 1000 ml

30 mg / l Rose Bengal was added to this culture medium and sterilized at 120 ° C for 15 minutes, and 30 mg / l streptomycin was added to prevent bacterial recurrence after cooling to approximately 45-50 ° C was anticipated (26).

2.2.1.1.B Potato Dextrose Agar

Potatoes (Peeled and sliced) 200 g

Dextrose 20 g

Agar 15 g

Water 1000 ml

The culture medium was prepared as described in the literature and autoclaved. After sterilization at 121 °C for 20 minutes at a pressure of 1.02 Atm / cm 2, the cold was expected and 30 mg / l streptomycin were added. Then pour 15-20 ml into sterile Petri dishes (26).

2.2.1.1.C Malt Extract Agar

The above materials are heated in distilled water. The materials are melted by mixing. Sterilized at 121 °C for 15 minutes. The pH is adjusted to 3. 5 to prevent bacterial replication. For this, 10% citric acid was used. 10-20% sucrose was added to the medium (26).

2.2.1.1.D Czapek's Agar

NaNO3 (Sodium nitrate) 2. 0 g

K2HPO4 (Di potassium phosphate) 1. 0 g

KCl (Potassium chloride) 0. 5 g

MgSO4. 7 H2O (Magnesium sulphate) 0. 5 g

FeSO4. 7 H2O (Iron sulphate) 0.01 g

Sucrose 30. 0 g

Agar 15. 0 g

Distilled water 1000 ml

The other salts are dissolved in 500 ml of water, except for salt in the formula of the K2HPO4. This mixed sugar is added, the K2HPO4 salt is melted separately and the prepared mixture is added. Then 1000 ml volume is completed and 15 grams of agar is added by melting, filtered and sterilized as above (26).

Malt extract 30. 0 g

Peptone (Mycological) 5. 0 g

Agar 15. 0 g

Distilled water 1000 ml

2.2.1.2 Preparation of preparations

Microscopic structures of microfungi were examined using picric acid-stained lactophenol solution and cotton blue actophenol solution. The lactophenol solution is prepared as outlined below. Cotton blue actophenol solution is used for distinquish genus, whereas the lactic acid solution with picric acid was used to make species determinations. Cotton blue paint the cell wall (26).

Lactic acid 20 g

Phenol 20 g

Glycerin 20 g

Distilled water 20 g

Saturated solution was prepared by adding picric acid to the medium and much of the picric acid was filtered. .The picric acid-stained and cotton-blotted lactophenol solution prepared as described above was used in the preparation of the preparation. These media were instilled with a drop on the slide and fruiting organs and mycelia of microfungi were transferred into the medium by sterile injection and covered with lamella. Then the sides of the coverslip were covered with nail polish (26).

2.2.1.3 Detection of microfungi

Preparations prepared from pure cultures were examined under microscope. The various organs of microfungi were measured 50 times and averaged. The diagnosis of microfungi was made by using local and foreign work. Olympus branded microscope was used in the study. An ocular micrometer was placed in the oocytes of the microscope to make the measurement (26).

2.2.2. Preparation of The Samples Before The Elisa Test

1) The samples were opened sterile and 25 gr sample is measured.

Figure 4: Preparation of black pepper samples.

Figure 5: Weighed samples

2) %60 methanol in the amount of 100 ml is added to samples.
3) The mixture is blended for 1 hour with a blander.

Figure 6: Blender

4) The mixture is filtered through a standart paper with Whatman no1.

Figure 7: Filtered extract from Whatman paper

5) Filtered liquids were injected through the colon (Figure 8).

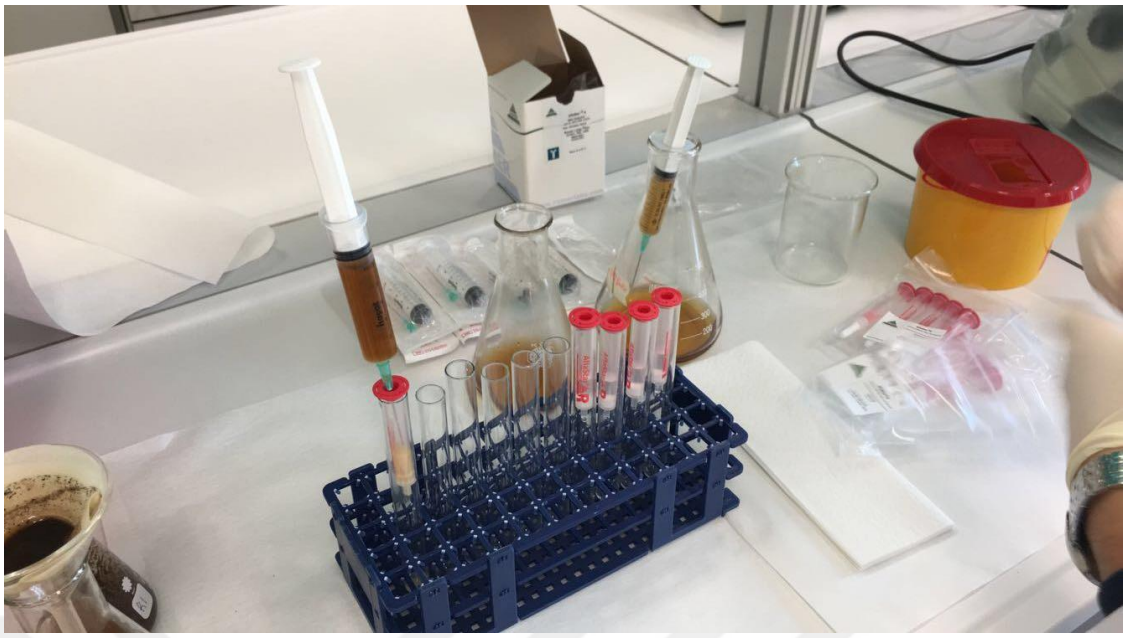

Figure 8: Injected through the colon

6) 10 ml of distilled water was injected through the columns two times by injection (Figure 9).

Figure 9: Distilled water was injected through the columns.

7) The wastewater is drained.

8) Clean test tubes were placed under the columns.

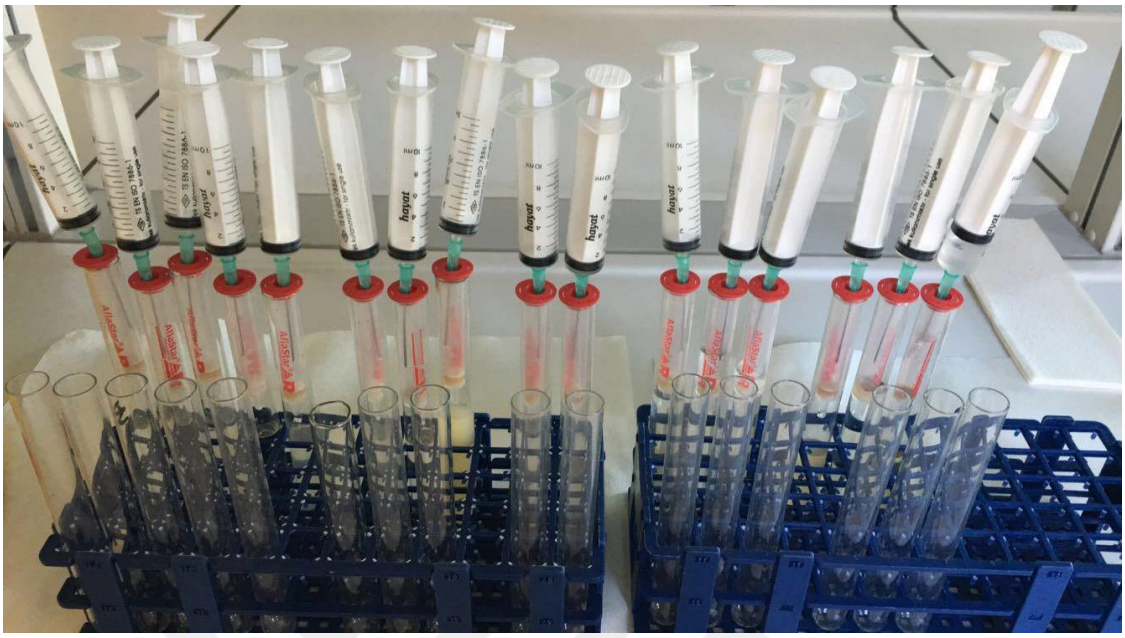

Figure 10: Clean test tubes

9) 1.5 (0.5x3) methanol is passed through the columns.
10) Than the analyte is used for analysis.

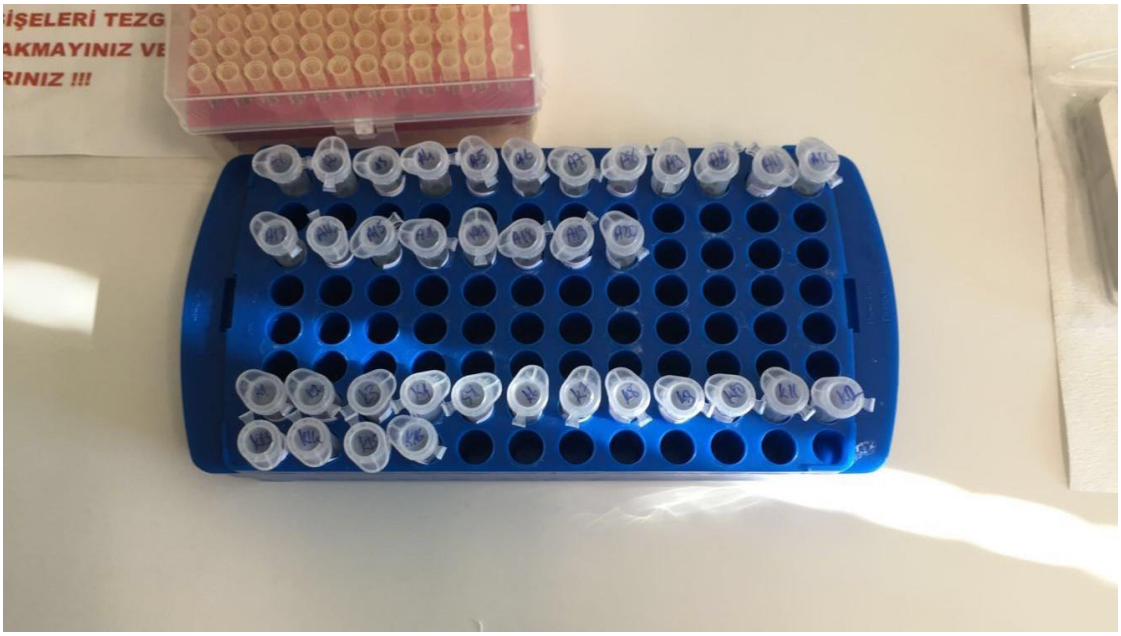

Figure 11: The analytes

2.2.3 Performing Elisa Test

1) Before test the kit bring to room temperature (20-25 °C / 68 – 77 °F).

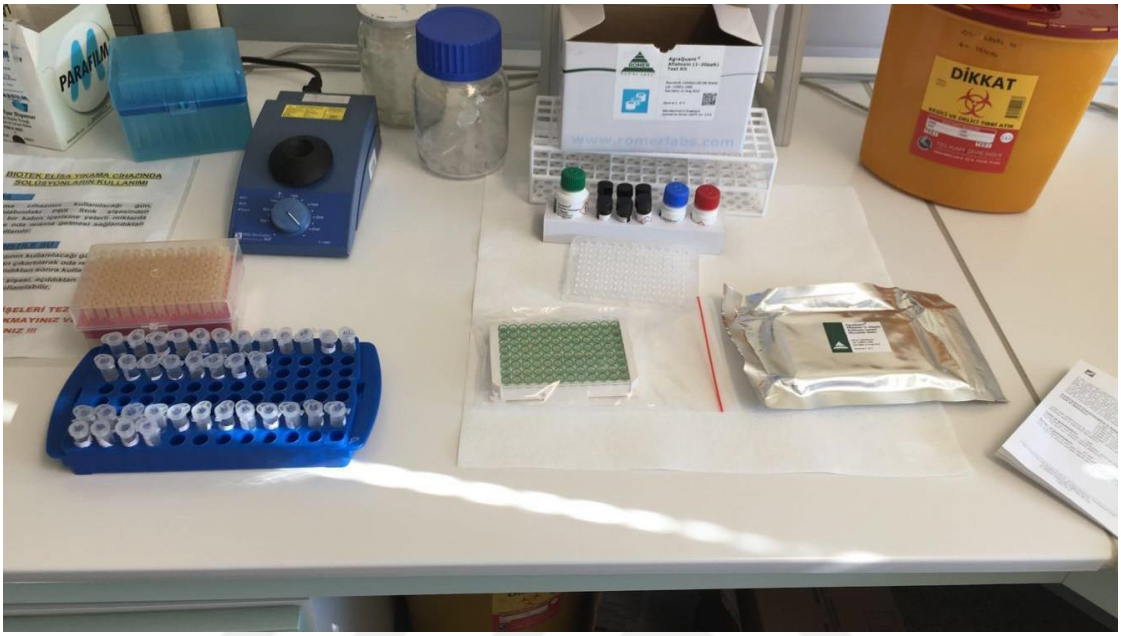

Figure 12: Romer Elisa kit

2) A sufficient number inserted of microtiter wells into the microwell holder for all standards and samples was duplicated in run. Record standart and sample positions.

3) Then put the 200 µl of conjugate into each dilution well with a multi-channel pipette.

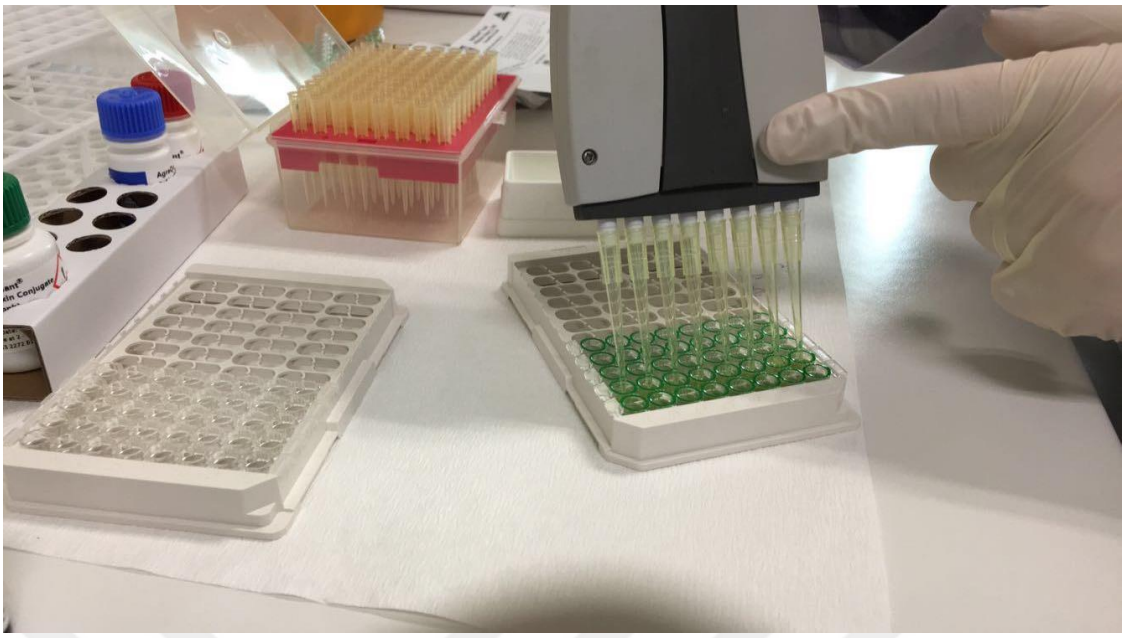

Figure 13: Dispencing Conjugate into each dilution well

4) Then 100 µl of standard and samples are pipetted into the respective wells (Figure 14).

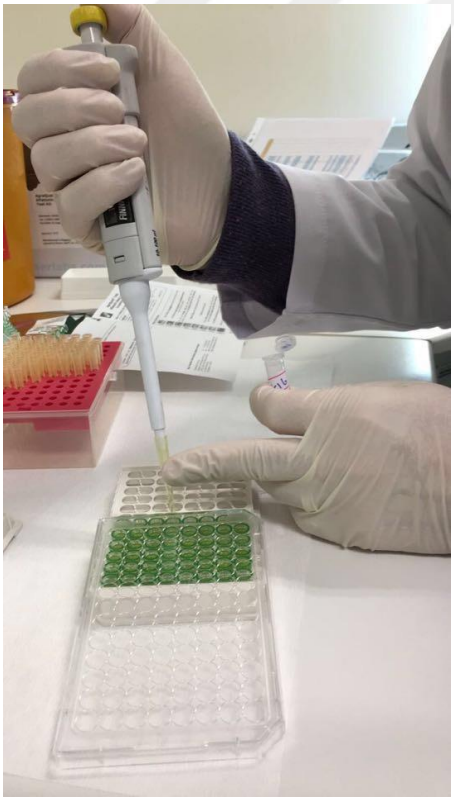

Figure 14: Adding standards and samples

5) The plate was mixed.

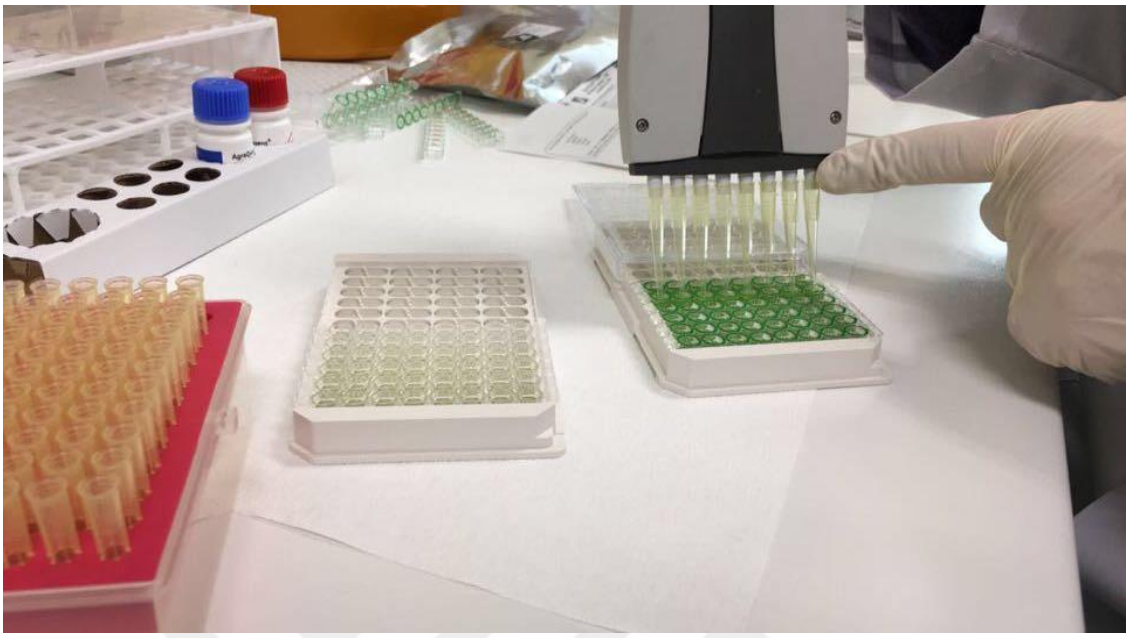

Figure 15: Pipetting it up and down 3 times

6) 100 µl of the contents from each dilution well into a corresponding Antibody coated microwell (Figure 16).

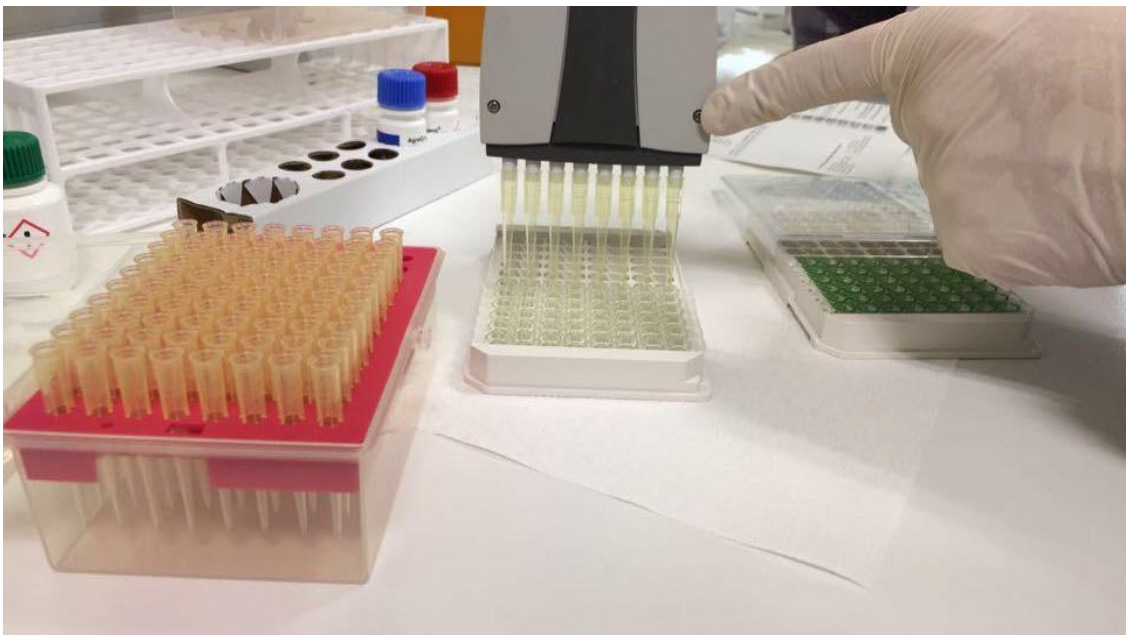

Figure 16: Transfering the contents from each Dilution well into antibody coated microwell

7) And incubated for 15 minutes in the dark at the room temperature (20-25 °C / 68 – 77 °F).

8) At the end of the incubation, the water in the wells was drained.

9) The solutions were washed 5 times with Biotek ELx50.

Figure 17: BioTek washer ELx50

11) 100 µl substrate was added to each each microwell strip using an 8-channel pippetor. Mixed gently by shaking the plate manually and incubated for 5 minutes in the dark at the room temperature (20-25 °C / 68 – 77 °F) (Figure 18).

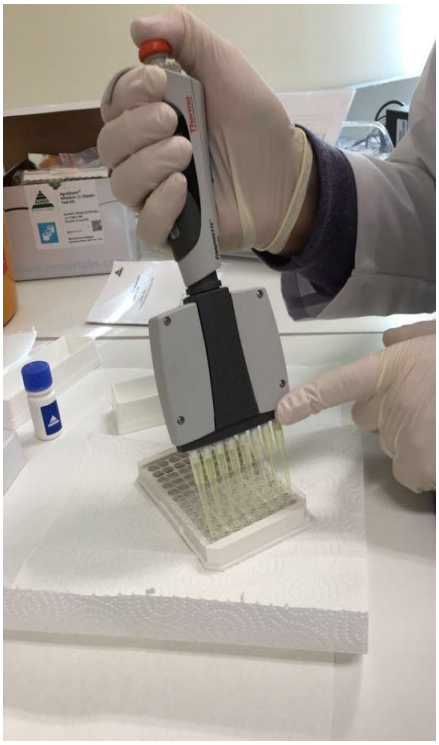

Figure 18: Pipetting substrate into each microwell

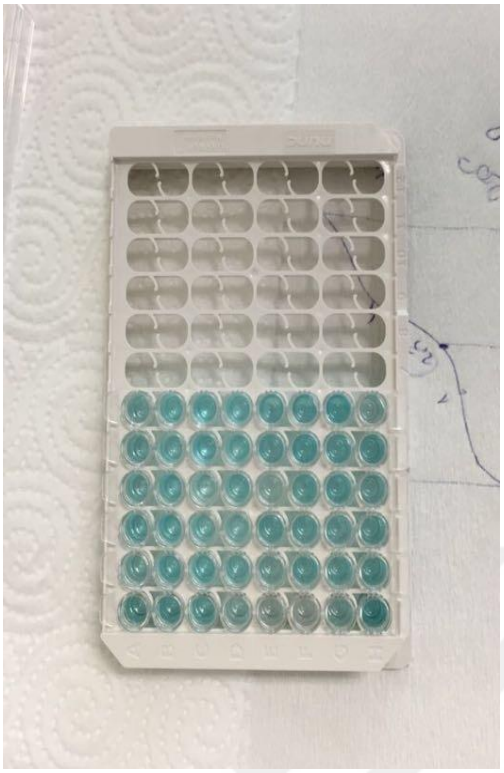

Figure 19: After incubation for 5 minutes

12) 100 µl stop solution was added to each microwell strip using an 8-channel pippetor. The plate manually mixed gently by shaking and measured the absorbance at 450 nm. After addition of stop solution readed within 30 minutes in Elisa reader (Figure 20).

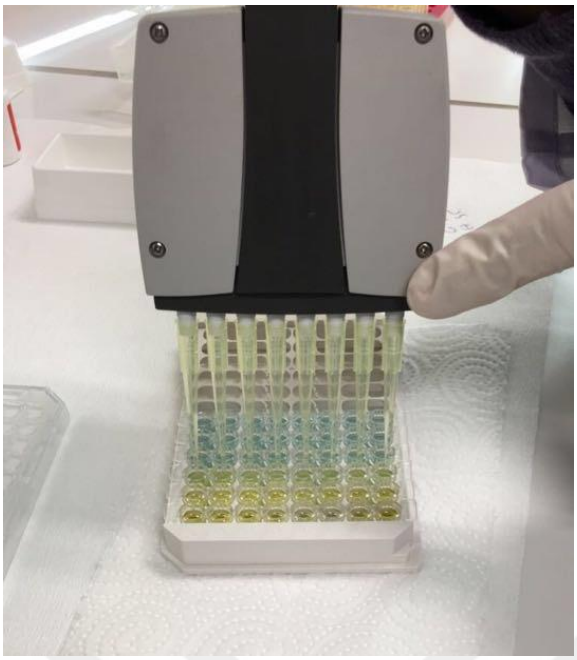

Figure 20: Pippeting stop solution into each microwell

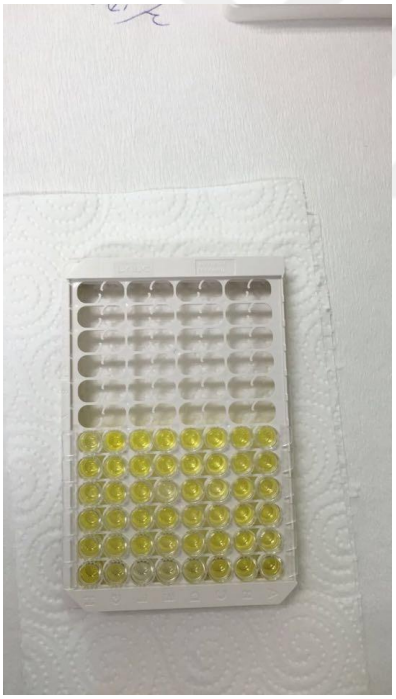

Figure 21: The color changing from blue to yellow

Figure 22: Placing microwell into the DTX 880 Elisa reader

Figure 23: Using 450nm filter

The aflatoxin Standard concentrates are provided in the following concentrations:

Standart 1: ……..0,7 ppb

Standart 2: …….....2 ppb

Standart 3: …….4 ppb

Standart 4:……10 ppb

Standart 5:……20 ppb

3. RESULTS AND DISCUSSION

When the Elisa plate which was being worked on was read in Elisa reader device, in order to check the accuracy of the test the Standard values and curve were reviewed (Figure 24).

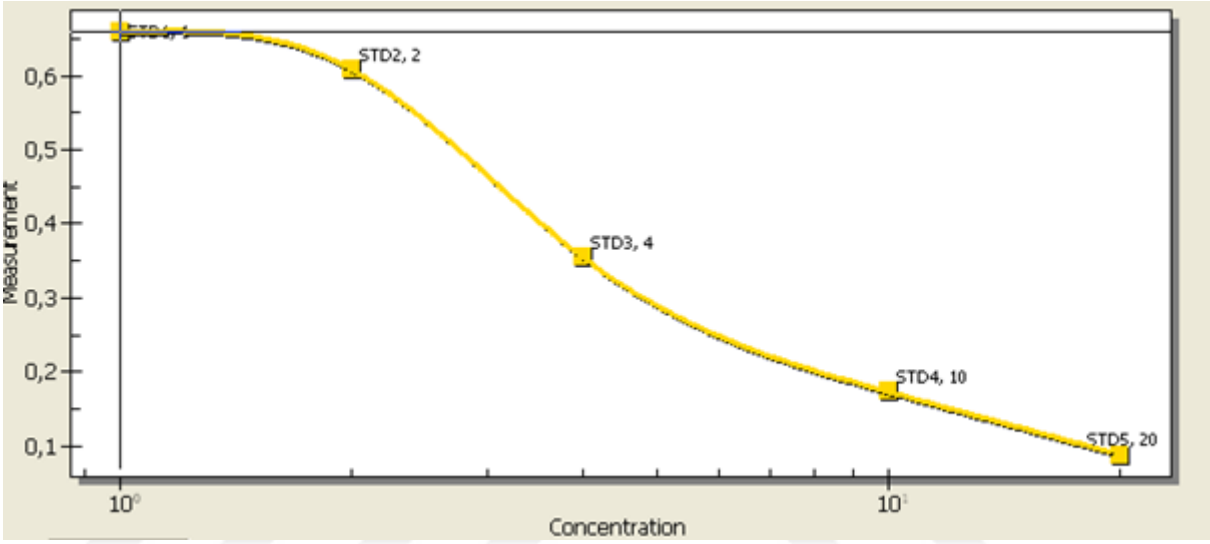

Figure 24 : Standard Curve

Standard values, wich were received from the Elisa reader device, observed as expected results. Than the test results were obtained as 3.928 - 0.453 ppb. Without one resulaflatoxin test results were found to be with the resonable bounds by the Turkish Food Codex. The results should be up to 10 ppb according to Turkish Food Codex, Europian regulations and, FDA. In the experiences performed in Turkey, it is being observed that the amount of Aflatoxin in black pepper is low.

When compared Elisa test results in packaged and unpackaged black pepper samples, there is no significant difference in the amount of aflatoxin.

Table 4 : Quentities of total aflatoxin in unpackaged black pepper sample

1	UNPACKAGED	2,955
2	UNPACKAGED	1,567
3	UNPACKAGED	1,566
4	UNPACKAGED	2,24
5	UNPACKAGED	3,452
6	UNPACKAGED	1,791
7	UNPACKAGED	2,34
8	UNPACKAGED	2,03
9	UNPACKAGED	1,759
10	UNPACKAGED	1,389
11	UNPACKAGED	2,868
12	UNPACKAGED	3,184
13	UNPACKAGED	1,934
14	UNPACKAGED	2,257
15	UNPACKAGED	2,889
16	UNPACKAGED	2,318
17	UNPACKAGED	2,818
18	UNPACKAGED	1,904
19	UNPACKAGED	3,928
20	UNPACKAGED	2,302

Table 5 : Quentities of total aflatoxin in packaged black pepper samples

1	PACKAGED	10,496
2	PACKAGED	2,269
3	PACKAGED	1,952
4	PACKAGED	2,874
5	PACKAGED	2,419
6	PACKAGED	2,432
7	PACKAGED	1,998
8	PACKAGED	2,471
9	PACKAGED	2,194
10	PACKAGED	0,487
11	PACKAGED	2,064
12	PACKAGED	0,703
13	PACKAGED	1,725
14	PACKAGED	0,453
15	PACKAGED	0,464
16	PACKAGED	0,599

According to the test results obtained, the lowest amount of aflatoxin is 0.453 ppb and the highest result is 10.496. The avarage aflatoksin amaount is 2.3 ppb.

Tablo 6: Isolated mold samples from unpackaged black pepper samples.

1	UNPACKAGED	No growth
2	UNPACKAGED	*Penicillum spp*
3	UNPACKAGED	*Penicillum spp*
4	UNPACKAGED	No growth
5	UNPACKAGED	No growth
6	UNPACKAGED	No growth
7	UNPACKAGED	No growth
8	UNPACKAGED	No growth
9	UNPACKAGED	No growth
10	UNPACKAGED	No growth
11	UNPACKAGED	No growth
12	UNPACKAGED	*Penicillum spp*
13	UNPACKAGED	No growth
14	UNPACKAGED	No growth
15	UNPACKAGED	No growth
16	UNPACKAGED	No growth
17	UNPACKAGED	No growth
18	UNPACKAGED	No growth
19	UNPACKAGED	No growth
20	UNPACKAGED	*Penicillum spp*

Tablo 7: Isolated mold samples from packaged black pepper samples

1	PACKAGED	No growth
2	PACKAGED	No growth
3	PACKAGED	No growth
4	PACKAGED	*Aspergillus niger*
5	PACKAGED	No growth
6	PACKAGED	No growth
7	PACKAGED	*Rhizopus spp*
8	PACKAGED	No growth
9	PACKAGED	No growth
10	PACKAGED	No growth
11	PACKAGED	No growth
12	PACKAGED	No growth
13	PACKAGED	*Rhizopus spp*
14	PACKAGED	No growth
15	PACKAGED	No growth
16	PACKAGED	No growth

We isolated pathogen microorganisms from 8 samples in 36 samples in total (22%). 78% of the samples are detected no-growth.

15 unpackaged black pepper samples and 13 packaged black pepper samples are detected no-growth. From 8 samples which pathogenic microorganisms isolated, 3 of them from packaged biack pepper samples, 5 of them from unpackaged black pepper samples.

We isolated Penicillium spp in 4 samples (11%), Aspergillus niger in 2 samples (5 %), Rhizopus sppin 2 samples (5%).

3.1 DISCUSSION

Black pepper is commonly consumed in every region of Turkey and has an important place in economy of the country. Due to their frequent occurrence and toxicity, guidelines and tolerance levels of aflatoxins have been set in several countries including Turkey. According to the Turkish Food Codex, the maximum residue limits for AFB1 and total aflatoxin in spices is 5 and 10 µg/kg, respectively (20). There are some studies on the level of mycotoxins in different foods consumed and produced in Turkey.

Freire *et al*., (2000) studied mycoflora and mycotoxins in Brazilian black and white pepper. Twenty metabolites were observed from black pepper, and seven from white pepper which were also detected in black pepper. *Chaetocin* and *penitrem A* were identified from the neutral fraction and *xanthocillin* from the acid fraction of black pepper. The toxicities of the metabolites were also studied. *Chaetocin* was cytostatic, *xanthocillin* was not known, tenuazonic acid inhibited plant growth, and *penitrem A* was tremorgenic (27).

Punam Jeswal and Dhiraj Kumar analysed nine different Indian spices (red chilli, black pepper, turmeric, coriander, cumin, fennel, caraway, fenugreek, and dry ginger) commonly cultivated and highly used in India. 56% *Aspergillus flavus* from red chilli and 45% *Aspergillus ochraceus* from black pepper were toxigenic and produced aflatoxins and *ochratoxin A*, respectively. *Penicillium citrinum* produced citrinin in red chilli, black pepper, coriander, cumin, fenugreek, and dry ginger samples. The highest amount of AFs was found in red chilli (219.6 ng/g), OTA was in black pepper (154.1 ng/g) (28).

In another study, Mendes at al. Twenty-two samples of pepper (4 black and 18 white) were analyzed for aflatoxins by high performance liquid chromatography with fluorescence detection. The degree of contamination in a lot of white pepper imported from India (six samples) and in sixteen samples randomly purchased from the market was estimated. Fourteen of the analyzed samples contained aflatoxins (B1, B2, G1, or G2); however, they were below the limit of 10 ppb fixed by the European Union, except for one (29).

Barani at al. reported that During the period from September to November 2014, occurrences of Aflatoxin B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) were determined in 76 pepper samples consisting black pepper (n = 40) and red pepper (n = 36) obtained from local markets of Isfahan province, Iran. Aflatoxins' (AFs') analyses were carried out by using the high-performance liquid chromatography (HPLC) method. AFB1 levels in 32 (88.9%) of 36 red pepper samples were higher than the Iranian and European maximum permitted level (>5 μg/kg). Total AFs were detected in 41 out of 76 samples (53.9%) while 25 pepper samples (32.9%) had levels of the toxin above the Iranian and European permitted level, that is, 10 μg/kg. This study shows that incidence of AFs' contamination in red pepper in Iran was significantly higher than black pepper ($p < .05$) (30).

Mozaffari Nejad at al. analysed 36 samples of spices from Iran and India that include chilli powder (n = 12), black pepper powder (n = 12) and whole black pepper (n = 12). Enzyme-linked immunosorbent assay was applied to analyse AFB_1in the samples. All the analyses were done twice. AFB_1 was found in all the spices samples, the concentration of AFB_1 in Iranian samples was ranged from 63.16 to 626.81 ng/kg and in Indian samples was ranged from 31.15 to 245.94 ng/kg. The mean of AFB_1 concentration in the chilli powder was significantly higher ($p < 0.05$) than the whole and powdered black pepper. However, none of the samples exceeded the maximum prescribed limit, that is 5000 ng/kg (5 μg/kg) of European Union regulations for AFB_1 (31).

Adzahan N at al. analysed 126 local and imported samples of commercial white and black pepper in Malaysia were analysed for aflatoxins B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2) content using high-performance liquid chromatography (HPLC) with a fluorescence detector (FD). An acetonitrile-methanol-water (17 : 29 : 54; v/v) mixture was used as a mobile phase and clean-up was using an immunoaffinity column (IAC). Seventy out of 126 (55.5%) samples were contaminated with total aflatoxins, although only low levels of aflatoxins were found ranging from 0.1 to 4.9 ng g(-1). Aflatoxin B1 showed the highest incidence of contamination and was found in all contaminated samples. There was a significant difference between type of samples and different brands ($p < 0.05$). The results showed black peppers were more contaminated than white peppers (32).

Delcourt A at al. analysed 20 random samples of pepper powders for contamination by microorganisms of public health significance (bacteria, moulds) and for aflatoxin B1 concentration. Black peppers were more heavily contaminated than white peppers but the most important bacteria causing foodborne diseases were not isolated; The mould flora was dominated by xerophilic species, particularly Aspergillus fumigatus, A. flavus, A. niger and A. ochraceus. These potentially pathogenic species can be dangerous for predisposed patients (33).

Mahhyastha and Bhat, studied the growth of *A.parasiticus* and production of aflatoxin on black and white pepper and found that black pepper supported fungal growth and aflatoxin production better than white pepper, the values being 62.5 µg/kg and 44 µg/kg respectively under laboratory conditions. In spite of these high aflatoxin values, researchers claim that both black and white pepper could be considered as poor substrates for fungal growth and aflatoxin production because they found that piperine and pepper oil inhibeted *A.parasiticus* growth and aflatoxin production (27).

Ferreira et al., studied 18 samples of white and 4 samples of black pepper imported from India. They used silica and c18 columns together which providd good clean up of pepper extracts for HPLC analysis, with sensitivity at the low $\mu g/kg^{-1}$ level. Only one white pepper sample was found to be heavily contaminated with aflatoxins (total aflatoxin > 20 µg/kg). Most of the analysed samples contained two of four aflatoxins, however, they were below the limit of 20 µg/kg fixed by the European Union. No aflatoxin was detected in one black pepper and seven white pepper samples (27).

Aziz and Youssef examined 130 spice samples used in meat products for aflatoxin and aflatoxigenic moulds in a study conducted in Egypt. Spice samples used in the investigation were collected from local meat-processing companes. Aflatovin B_1 was detected in four samples of black pepper (35 µg/kg) and four of white pepper (22 µg/kg). The most commonly isolated moulds were *Aspergillus flavus* (24 isolates) and *A. Parasiticus* (16 isolates). Aflatoxin contamination of processed meat was found to be correlated with the addition of spices to fresh meat ingredients (27).

In our study; the tests result were obtained as ≥0.453 - 3.928 ppb (Table 4-5). Except for one all aflatoxin results were found to be with the resonable bouds by the Turkish Food Codex. The results are should be up to 10 ppb according to Turkish Food Codex, European regulations and FDA. The experiences performed in Turkey, it is being

observed that the amount of Aflatoxin in black pepper is low. So, the detection of aflatoxin must be a necessary criterion to be added to microbial criteria to appreciate the quality of peppers.

Hilal Çolak at al., analysed 84 samples of spices (30 red-scaled pepper, 30 red pepper and 24 black pepper) commercialized in Istanbul were randomly obtained from markets and bazaars. 36 out of 84 spice samples (42.9%) were found to be contaminated with aflatoxins in the range of 0.3-46.8 µg/kg. The levels of total aflatoxins were determined to be ranging from 0.3-16.7 µg/kg in 8 out of 24 black pepper samples. Only two samples contained AFB1 at the levels of 9.8 and 10.3 µg/kg (34).

Erol at al., analysed 25 black pepper samples. They found that 76 % of black pepper had enterobacteriaceae. B. cereus was isolated from 80 % of black pepper. Numbers of total mesophilic aerobic count were 4.4x103 cfu/g for black pepper (35).

As a results, the amount of mycotoxin in food is a quality criterion in terms of nutrients. The hazards that may arise from aflatoxins should be prevented at the beginning. To minimize the aflatoxin risk storage and packaging of nutrients should be successfully achieved. The compounds which leads to reproduction of aflatoxin need a certain humidity and temperature. The nutrients which are not dried well and exposed to moist atmosphere mostly produce aflatoxin-like substance.

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5. CIRRICULUM VIRTAE

Kişisel Bilgiler

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Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora			
Yüksek Lisans	Beslenme ve diyetetik	**Yeditepe üniversitesi**	2017
Lisans	Beslenme ve diyetetik	**Yeditepe üniversitesi**	2014
Lise	sayısal	**Özel safiye sultan lisesi**	2008

Bildiği Yabancı Dilleri	Yabancı Dil Sınav Notu
ingilizce	-

Staj Deneyimi

Görevi	Kurum	Süre
Stj. Diyetisyen	GATA	Ocak 2014 - Haziran 2014
Stj. Diyetisyen	Zeynep kamil çocuk ve kadın hastalıkları hastanesi	Kasım 2013 - Ocak 2014
Stj. Diyetisyen	Kanuni sultan Süleyman eğitim v araştırma hastanesi	Ekim 2013 - Kasım 2013
Stj. Diyetisyen	Çapa tıp fakültesi hastanesi	Eylül 2013 - Ekim 2013
Stj. Diyetisyen	Bahçelievle medical park	Haziran 2013

Bilgisayar Bilgisi

Program	Kullanma becerisi
Microsoft Office word	İyi
Microsoft Office PowerPoint	İyi