



Investigation of Natural Mycoflora and Aflatoxin Formation in Hazelnuts and Products

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Abstract. In this study, natural mycoflora of 30 raw and 50 roasted hazelnut, 20 hazelnut paste and 50 inner membrane samples and their total aflatoxin contents were determined. In mycological isolations, 1.8-2.56% of *Aspergillus flavus* and 42.7-65.44% of *A. niger* were determined in the raw hazelnut samples. *A. flavus* (2.2-12.2%) and *A. niger* (33.3-74.5%) were also detected in roasted hazelnut, while the percentages of these microorganisms in hazelnut paste samples were 0-13.1% and 43.5-100.0%, respectively. The ratio of *A. flavus* and *A. niger* in inner membranes was found to be 2.6-16.2% and 44.6-89.4%, respectively. Aflatoxin analysis showed that the levels of aflatoxin were 2.11–10.03 ppb in raw hazelnut, 0.1–4.04 ppb in roasted hazelnut, 0.2-6.02 ppb in hazelnut paste samples and 0.7-38.2 ppb in inner membrane samples. While only one of the raw hazelnut, roasted hazelnut and hazelnut paste samples had toxin above the legal limit, 100% of the inner membrane samples showed different levels of aflatoxin contamination. Since there is no limitation in the Turkish Food Codex on hazelnut inner membrane, the amount of aflatoxin, which is higher than 10 ppb in 25 of the samples, was considered to be high contamination. Kruskal Wallis and Mann Whitney U analyses were used for statistical evaluation of the samples. There was a significant difference in aflatoxin formation and moisture content between the samples in different groups ($p < 0.05$).

Keywords: Aflatoxin, hazelnut, hazelnut products, mycoflora.

Fındık ve Fındık Ürünlerinde Doğal Olarak Oluşan Mikoflora İle Aflatoksin Oluşumlarının Araştırılması

Özet. Bu çalışmada, 30 çiğ, 50 kavrulmuş fındık, 20 ezme ve 50 iç zar örneğinde oluşan doğal mikoflora ve toplam aflatoksin içerikleri belirlenmiştir. Yapılan mikolojik izolasyonlarda çiğ fındıkta %1,8-2,56 *Aspergillus flavus*, %42,7-65,44 *A. niger*; kavrulmuş fındıkta %2,2-12,2 *A. flavus*, %33,3-74,5 *A. niger*; ezme örneklerinde %0-13,1 *A. flavus*, %43,5-100 *A. niger*; iç zarda ise %2,6-16,2 *A. flavus*, %44,6-89,4 *A. niger* belirlenmiştir. Aflatoksin analizlerinde çiğ fındıkta 2.11–10.03 ppb, kavrulmuş fındıkta 0,1–4,04 ppb, ezme örneklerinde 0,2-6,02 ppb ve iç zar örneklerinde ise 0,7-38,2 ppb seviyelerinde aflatoksin içerikleri saptanmıştır. Çiğ fındık, kavrulmuş fındık ve ezme örneklerinin sadece 1'inde yasal sınırın üzerinde toksin içeriği bulunurken, iç zar örneklerinin % 100'ünde değişik seviyelerde aflatoksin bulaşıklığı bulunmuştur. Fındık iç zarı ile ilgili Türk Gıda Kodeksinde bir sınırlama olmadığı için örneklerin 25'inde 10 ppb'den yüksek olan aflatoksin oluşumları yüksek bulaşıklık olarak değerlendirilmiştir. Çalışılan örneklerin istatistikî değerlendirmelerinde Kruskal Wallis ve Mann Whitney U analizleri kullanılmıştır. Farklı gruptaki örnekler arasında aflatoksin oluşumu ve nem içerikleri arasındaki değişim istatistikî açıdan önemli bulunmuştur ($p < 0.05$).

Anahtar Kelimeler: Aflatoksin, fındık, fındık ürünleri, mikoflora.

1. INTRODUCTION

Hazelnut (*Corylus avellana* L.) forms the basis for the more important commercial cultivars and is belonging to *Betulaceae* family. The hazelnut (*Corylus avellana* L.) forms the more important commercial cultivars. *C. maxima* and *C. colurna* (Turkish hazel) are also common others. Hazelnut is grown in the Blacksea Region of Turkey, especially in Trabzon, Ordu, Giresun and Akçakoca. Although vary depending on the year, Turkey ranks the first in the world with approximately 600 thousand tonnes of hazelnut production [1].

Hazelnut is a food rich in proteins, lipids, carbohydrates, vitamins, minerals, dietary fibers, tocopherols, phytosterols and phenolic compounds [2,3]. Monounsaturated fatty acids (especially oleic acid) and polyunsaturated fatty acids in hazelnut have been reported to have beneficial effects on human health [3]. Hazelnut oil has been found to reduce cholesterol levels in the blood [4]. Hazelnut contains tocopherols, phytosterols, squalene and phenolic compounds that help prevent chronic diseases such as cancer and cardiovascular diseases [5,6].

There are biological, chemical and microbiological factors limiting hazelnut production. The most important microbiological factors limiting hazelnut production are fungal contamination and aflatoxins, one of the metabolites produced by these fungi. Aflatoxins are secondary metabolites produced by *Aspergillus* species. To date, more than 20 different aflatoxins have been reported. However, B₁, B₂, G₁, G₂ were reported to be four naturally occurring main forms. *A. flavus* produces only AFB₁ and B₂, while *A. parasiticus* produces AFB₁, B₂, G₁ and G₂ [7-9].

Aflatoxins produced by *Aspergillus* spp. in field conditions, transportation, processing and storage can be found in oilseeds such as nuts, pistachios, peanuts, almonds, sunflowers, in cereals and cereal products, pulses, spices, milk, dairy products, meat, meat products and animal products such as eggs [10-12].

Aflatoxins have been reported to have several effects on human and animal health such as mutagenic, carcinogenic, teratogenic (embryonal damage), tremorogenic (problems of tremor and reflex loss), hemoragic (tissue and organs bleeding problems), dermatitic (skin lesions), hepatotoxic

(liver damage), nephrotoxic (kidney system damage), and neurotoxic (nervous system damage) [13-15]. Aflatoxins are known to be the most effective hepatocarcinogen in many animal species. In many countries, aflatoxins are included in the class 1A carcinogens by the International Organization for Cancer Research, since there is a positive correlation between consumption of aflatoxin-contaminated food and an increased risk of liver cancer [16].

The maximum limits of aflatoxins in many foodstuffs have been established and published in each country. In order to protect public health, importing countries, especially the European Union countries, aim to reduce the legal values of aflatoxins to zero in risky products. To this end, aflatoxin B₁ limit in many countries has been reduced from 5 ppb to 2 ppb and the total aflatoxin (B₁+B₂+G₁+G₂) in hazelnuts has been reduced from 10 ppb to 4 ppb (Commission Regulation EC No 194/97). In the Turkish Food Codex, the total aflatoxin content in hazelnut and its products is determined to be 10 ppb and Aflatoxin B₁ content is maximum 5 ppb [17,18].

As mentioned above, although vary depending on the year, Turkey ranks first in the world with approximately 600 thousand tonnes of hazelnut production [1]. In addition, Aflatoxin contamination is one of the most important problems in hazelnut and hazelnut products. Due to the aflatoxin values in nuts exported by Turkey are sometimes above the legal limits, these products are rejected by the countries, which leads to huge economic losses. Additionally, producers and industrialists are struggling economically, Turkey's reputation in foreign trade is damaged and marketing problems arise.

After the occurrence of aflatoxin, which is a problem in food all over the world, they cannot be controlled [19-21]. Therefore, the most effective method for the formation of aflatoxins produced by fungi is to harvest, dry, store and process food under conditions that reduce the risk of contamination and limit the development of fungi. These conditions can only be achieved by determining the natural mycoflora that is formed in the product and can cause toxin formation.

In this study, it was aimed to determine the naturally occurring mycoflora in raw hazelnut,

roasted hazelnut, hazelnut paste and hazelnut inner membrane and to investigate the total aflatoxin formation in these products.

2. MATERIALS AND METHODS

2.1. Supply of Hazelnut Samples

The samples used in the study were obtained from various factories and retail stores in Trabzon, Ordu, Giresun and Akçakoca regions where hazelnut production is intense in the Blacksearegion. In the sampling, raw hazelnut (n = 30), roasted hazelnut (n = 50), hazelnut paste (n = 20), and inner membrane (n = 50) samples were used. The hazelnut samples were provided immediately after harvest, the inner membrane samples were obtained during the drying period and the hazelnut paste samples were provided during the processing stages of the product. Samples were brought to the laboratory under appropriate conditions and kept in labelled cloth bags and refrigerated at 4 °C until tested.

2.2. Determination of Moisture Content of Samples

The moisture content of the hazelnut, hazelnut paste and membrane samples used in the study was determined by a precision balance and halogen lamp moisture analyzer according to the method reported by Bakker [22]. 5.0 ± 0.1 g of raw and roasted hazelnut samples were taken and shredded in hand blender and moisture contents were determined at 140 °C. In order to determine the moisture content of hazelnut membrane and hazelnut paste samples, 1 g of each sample was weighed directly without any process and evaluated according Özkaya [23] equation (1).

$$\% \text{Moisture content} = \frac{M_1 - M}{M_1 - M_0} \quad (1)$$

M_0 : Weight of container (Tare) (g)

M : Weight of the container and sample after drying

M_1 : Weight of sample with container

2.3. Mycological Isolations

Raw hazelnut, roasted hazelnut and inner membrane samples were sterilized with 2.0% (v/v) NaOCl solution and washed with distilled water. After surface sterilization, of dried hazelnut, roasted hazelnut and inner membrane samples, hazelnut paste samples were added directly to Potato Dextrose Agar (PDA). After surface sterilization was performed, raw hazelnut, roasted hazelnut and inner membrane samples were dried and then planted in PDA while surface sterilization

process was not applied for the cultivation of hazelnut paste samples. A total of 100 plantings were made for each sample using 10 petri dishes. Cultivated petri dishes were incubated at 24 °C for 4-5 days and thus colony development was ensured. At the end of incubation, the fungal colonies were differentiated based on macroscopic criteria such as size, colour, surface or overhead development, and colonies showing different growth were purified by classifying as *Fusarium* (F1, F2,...), *Aspergillus* (A1, A2, ...) and *Penicillium* (P1, P2,...).

After purification, single spore isolation was made on water and Czapek Dox Agar. Single spore isolates were incubated at 25 °C for 7 days by transferring them to a suitable growth medium. Cultures were examined under microscope during the incubation and general morphological definitions were made, colony number and characteristics were recorded.

Specific diagnostic keys have been used for the fungi species identified intensively as a result of mycological isolations. While the diagnostic keys developed by Raper and Fennel [24] and Samson and Pitt [25] is used for the identification of *Aspergillus* species, *Penicillium* species were identified according to the diagnostic keys of Samson et al. [26] and Pitt [27]. In identifying *Fusarium* and other species, diagnostic key developed by Barnett and Hunter [28] was used. In species identification of fungi; growth rates at certain temperatures (colony diameter and properties), overhead mycelium formation (colour and structure), the presence or absence of chlamidospores, sporodiosium development, fialitic properties, conidial cap (color and shape), conidial sequencing, colony colours, presence or absence of sclerotes, and presence of macro and micro conidia were evaluated.

2.4. Aflatoxin Analysis

CD-ELISA (Competitive Direct Enzyme-Linked Immuno Sorbent Assay) method was used to investigate the total amount of aflatoxin in raw hazelnut, roasted hazelnut, inner membrane and hazelnut paste samples. 5 different aflatoxin standards were used in CD-ELISA at 0, 1, 2, 4 and 8 ppb levels. Samples were prepared and extracted according to the procedure of Neogen Veratox®. CD-ELISA results were obtained by reading at 650 nm in a microwell reader. Veratox Software for Windows, Log/Logit and Single Test Format v3.02 software were used to calculate the toxin values in ppb.

3. RESULTS AND DISCUSSION

Moisture measurement analyzes were carried out to determine how moisture content of raw hazelnut, roasted hazelnut, inner membrane and hazelnut paste affect natural mycoflora and aflatoxin formation in these samples.

The moisture content of samples obtained from Trabzon, Ordu, Giresun and Akçakoca regions are given in Figure 1.

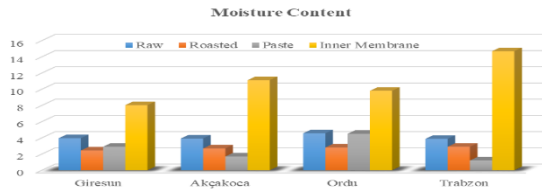


Figure 1. Moisture content of samples obtained from different regions

As can be seen from Figure 1, the moisture values of the raw hazelnuts were determined as 3.88-4.56%. It is recommended that the maximum moisture content in raw hazelnuts should be max. 4.5% [29]. It was observed that the moisture values of our samples were close to or higher than the recommended maximum value. Aluç and Aluç [30]

found the moisture values of 186 Akçakoca, 177 Ordu and 160 Giresun raw hazelnut samples to be 4.2-6.8%. According to meteorological data, the rainfall of the 4 regions is approximately 61.6-75.9 mm per year. Samples of raw nuts with the highest moisture content were obtained from Ordu region. This is due to the fact that the average annual rainfall in the Ordu region is generally higher than the other three regions.

As a result of mycological isolation of raw hazelnut, roasted hazelnut, hazelnut paste and inner membrane samples, *Aspergillus* species were observed as the most dominant fungal ones. Natural mycoflora determined as a result of mycological isolations in samples obtained from different regions is presented in Table 1 and Figure 2. *Aspergillus* and *Penicillium* species are predominant in the studies conducted on the determination of raw and roasted hazelnut mycoflora, however, *Rhizopus*, *Fusarium*, *Cladosporium*, *Trichothecium*, *Mucor*, *Alternaria* and *Trichoderma* species have also been reported to be isolated [31, 32]. No studies have been found in the literature on the determination of mycoflora in hazelnut paste and inner membrane samples.

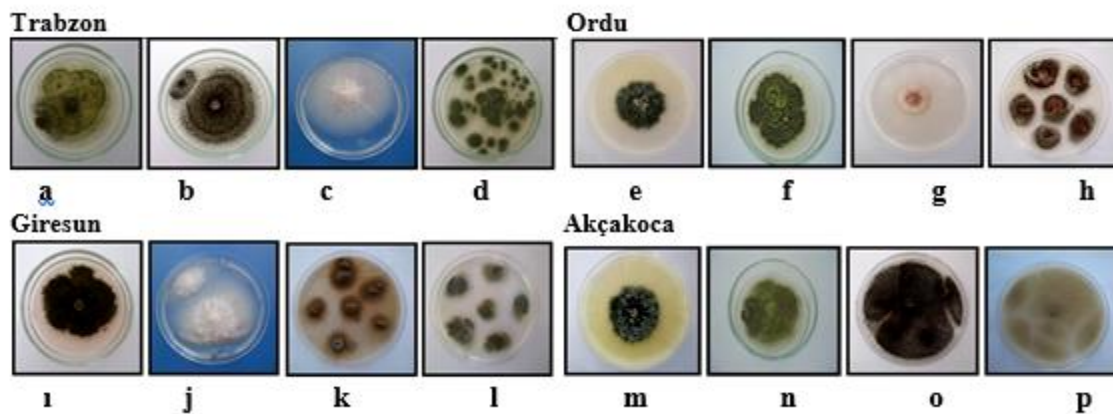


Figure 2. Isolated fungal species from sample groups. **a.** *A. flavus* from raw nuts; **b.** *A. niger* from roasted nuts; **c.** *Fusarium* spp. from hazelnut butter; **d.** *Penicillium* spp. from the inner membrane; **e.** *Trichoderma* spp. from raw nuts; **f.** *A. flavus* from roasted hazelnuts; **g.** *Fusarium* spp. from hazelnut butter; **h.** *A. niger* isolated from the inner membrane **i.** *A. flavus* from raw nuts; **j.** *Fusarium* spp. from roasted hazelnuts; **k.** *Trichoderma* spp. isolated hazelnut butter; **l.** *Penicillium* spp. isolated from inner membrane; **m.** *Trichoderma* spp. from raw nuts; **n.** *A. flavus* from roasted hazelnuts; **o.** *A. niger* from hazelnut butter; **p.** *Trichoderma* spp. from the inner membrane.

Figure 2. Isolated fungal species from sample groups.

Table 1. Natural mycoflora determined as a result							
Regions	Sample Type	Fungus Species	% Isolation	Regions	Sample Type	Fungus Species	% Isolation
Trabzon	Raw	<i>Aspergillus</i>	68.0	Ordu	Raw	<i>Aspergillus</i>	63.0
		<i>Penicillium</i>	19.2			<i>Penicillium</i>	23.3
		<i>Rhizopus</i>	12.8			<i>Rhizopus</i>	9.4
	Roasted	<i>Aspergillus</i>	57.8			<i>Fusarium</i>	2.6
		<i>Penicillium</i>	26.6			<i>Trichoderma</i>	1.7
		<i>Fusarium</i>	8.4			Roasted	<i>Aspergillus</i>
		<i>Alternaria</i>	4.6		<i>Penicillium</i>		33.5
	<i>Rhizopus</i>	2.6	<i>Fusarium</i>		16.5		
	Butter	<i>Aspergillus</i>	50.3		<i>Rhizopus</i>	5.5	
		<i>Penicillium</i>	43.5		Butter	<i>Aspergillus</i>	51.0
		<i>Fusarium</i>	6.2			<i>Penicillium</i>	20.6
	Inner Membrane	<i>Aspergillus</i>	60.6			<i>Rhizopus</i>	18.0
		<i>Penicillium</i>	33.0		<i>Fusarium</i>	10.4	
		<i>Rhizopus</i>	4.0		Inner Membr.	<i>Aspergillus</i>	95.0
		<i>Trichoderma</i>	1.4			<i>Penicillium</i>	4.4
	Giresun	Raw	<i>Aspergillus</i>			45.0	<i>Rhizopus</i>
<i>Penicillium</i>			21.0	Raw	<i>Aspergillus</i>	54.2	
<i>Rhizopus</i>			21.0		<i>Penicillium</i>	14.6	
<i>Fusarium</i>			13.0		<i>Rhizopus</i>	14.6	
Roasted		<i>Aspergillus</i>	78.5		<i>Fusarium</i>	8.3	
		<i>Penicillium</i>	17.5		<i>Trichoderma</i>	8.3	
		<i>Fusarium</i>	4.0		Roasted	<i>Aspergillus</i>	45.2
Butter		<i>Aspergillus</i>	63.7	<i>Rhizopus</i>		27.0	
		<i>Penicillium</i>	23.7	<i>Penicillium</i>		18.3	
		<i>Trichoderma</i>	10.0	<i>Fusarium</i>		9.5	
		<i>Rhizopus</i>	2.0	Butter	<i>Aspergillus</i>	81.3	
		<i>Fusarium</i>	0.6		<i>Penicillium</i>	18.7	
Inner Membrane		<i>Aspergillus</i>	4.8	Inner Membrane	<i>Aspergillus</i>	85.1	
		<i>Penicillium</i>	34.4		<i>Penicillium</i>	9.3	
		<i>Trichoderma</i>	12.2		<i>Trichoderma</i>	4.7	
		<i>Rhizopus</i>	5.6		<i>Rhizopus</i>	0.9	

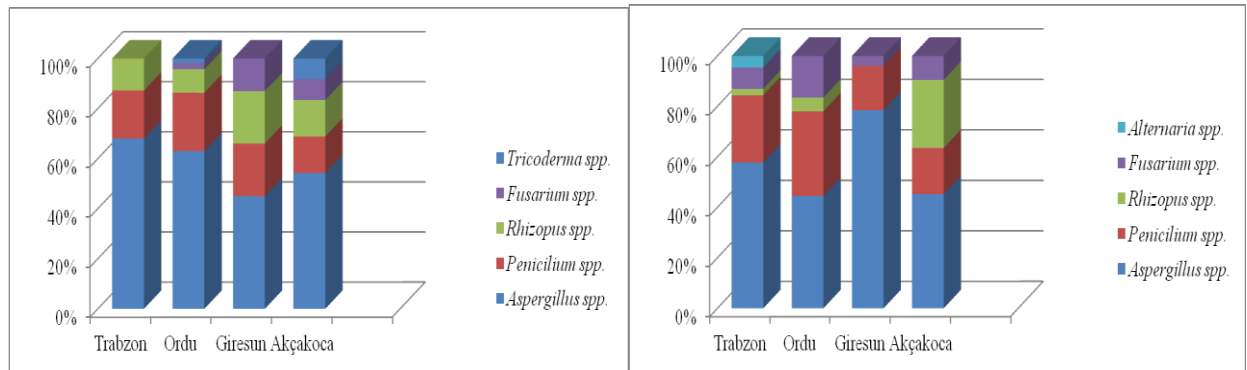


Figure 3. Fungus genus isolated from raw and roasted hazelnut samples.

Mycoflora in raw hazelnut was determined to be 45-68% of *Aspergillus* spp., 14.6-23.3% of *Penicillium* spp., 9.4-21% of *Rhizopus* spp., 2.6-13.0% of *Fusarium* spp., and 1.7-8.3% of *Trichoderma* spp. These ratios were found to be as follows in the mycoflora of roasted hazelnuts: 44.5-78.5% *Aspergillus* spp., 17.5-33.5% *Penicillium* spp., 2.6-27.0% *Rhizopus* spp., 4.0-16.5% *Fusarium* spp., 0.0-4.0% *Alternaria* spp. (Figure 3).

Various studies on the determination of natural microflora in raw and roasted hazelnuts yield similar results to the present study. In these studies, although *Aspergillus* and *Penicillium* species were dominant, *Rhizopus*, *Fusarium*, *Cladosporium*, *Trichothecium*, *Mucor*, *Alternaria* and *Trichoderma* species were also isolated [31-34]. *Aspergillus* spp. was found to be high in natural mycoflora of raw and roasted hazelnuts in many studies [35-37]. Other fungi genus and their proportions isolated from raw nuts are as follows: 12.8-23.3% *Rhizopus* spp., 9.4-19.2% *Penicillium* spp., 2.6-13% *Fusarium* spp. and 1.7-8.3% *Trichoderma* spp. According to a study carried out by Demir et al. [38] on 30 raw hazelnut samples, the most commonly isolated fungi genus have been reported as follows: 2.2- 100.0% *Aspergillus* spp., 4.0-67.4% *Penicillium*, 2.0-62.0% *Rhizopus*, 2.0-82.0% *Mucor* and 2.0-56.0% other fungus genera. Simsek et al. [39], identified 96.6% *Aspergillus* spp., 93.3% *Penicillium*, 96.6% *Rhizopus* and 83.3% *Mucor* in 30 hazelnut samples obtained from Giresun. It is thought that the reason of high observation of the *Aspergillus* spp. in many of our

samples is that the hazelnut was exposed to fungal contamination in the tree and during harvest. Furthermore, this indicates that although the product is harvested early and the shell forms a protective layer, there is a risk of contamination from cracks that may occur during harvest. In addition, high moisture content of hazelnut growing areas is one of the important factors that promote the development of *Aspergillus* spp.

According to our literature search could ascertain, no study is available on natural mycoflora formed in hazelnut inner membrane and hazelnut paste. However, it is thought that the data obtained from this study will serve as an example for other scientific studies in the future. As can be seen from Figure 4, 47.8-95.0% *Aspergillus* spp., 4.4-34.4% *Penicillium* spp., 1.4- 12.2% *Trichoderma* spp., and 0.6-4.0% *Rhizopus* spp. was determined in the inner membranes of hazelnuts samples collected from 4 regions. Unidentified fungi species were ignored. From hazelnut paste samples, 50.3-81.3% *Aspergillus* spp., 18.7-43.5% *Penicillium* spp., 2.0-18.0% *Rhizopus* spp., 0.0-10.0% *Trichoderma* spp. and 0.6-10.4% *Fusarium* spp. were isolated.

Although *Aspergillus* spp. species that can synthesize aflatoxin in hazelnut paste samples are different according to regions, they have been isolated and identified as the most dominant species in the present study by 50.3-81.3%. *Penicillium*, *Rhizopus*, *Fusarium* and *Trichoderma* strains were isolated in 18.7-43.5%, 2.0-18.0%, 0.6-10.4% and 0.0-10.0%, respectively in mycoflora of hazelnut paste samples.

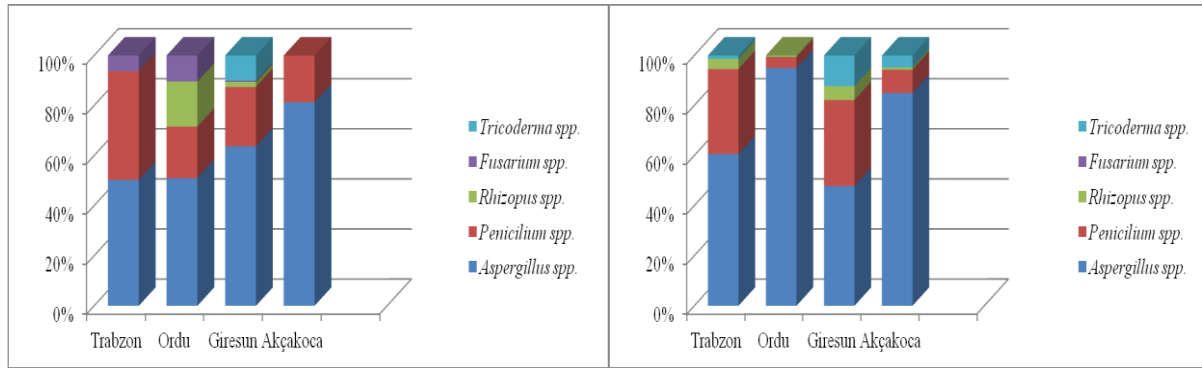


Figure 4. Fungus genus isolated from hazelnut paste and inner membrane samples.

The high rate of *Aspergillus* spp. species in most of our samples suggests that hazelnut is exposed to fungal contamination in the tree and during harvest.

This is because the development of *Aspergillus* spp. species in shelled hazelnuts starts on the surface of the shell when it is still in the tree, and it can increase during the contact of the product with the soil and harvesting. *Aspergillus* spp. are not seen in shelled hazelnuts that come into contact with the ground, while *A. flavus-parasiticus* develops in the hazelnut which is damaged in the

outer shells [31]. This is because the hard shell of hazelnut is a good protector against fungal contamination and bacteria.

50% of the total 150 raw hazelnuts, roasted hazelnuts, hazelnut paste and inner membranes, aflatoxin contamination was found to be at levels of 0.1-38.2 ppb. Aflatoxin levels of 2.11-10.03 ppb were determined in 9 of 30 raw hazelnut samples and only one sample had aflatoxin content above the legal limit. Aflatoxin contamination (14.0%) and aflatoxin levels (0.1-4.04 ppb) were below the legal limit in roasted hazelnut samples (Table 2).

Table 2. Toxin contents and contamination rates determined in the samples

Sample	Number of Samples	Aflatoxin (ppb)	Contaminated Samples	Exceeding Legal Limit	% Contamination
Raw	30	2,11-10,03	9	1	30
Roasted	50	0,1-4,04	7	-	14
Hazelnut Butter	20	0,2-6,02	9	-	45
Inner Membran	50	0,7-38,2	50	25	100

As a result of statistical evaluations between the moisture contents and aflatoxin formation of 4 different hazelnut samples obtained from different

regions, the relationship between moisture content and aflatoxin formation was found to be significant ($p < 0.05$) (Figure 5).

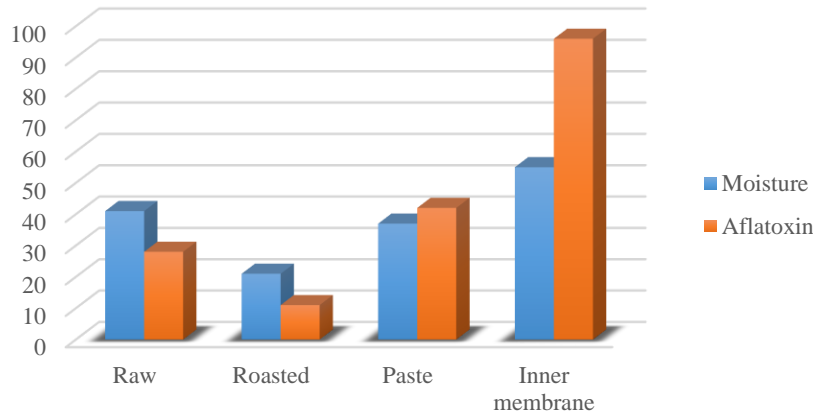


Figure 5. The relationship between moisture content and aflatoxin formation.

While the amount of aflatoxin was found to be 30% in the studied hazelnut samples, in only one of the samples obtained from 4 regions, total aflatoxin levels exceeded 10 ppb, which is the legal limit of aflatoxin determined by the Turkish Food Codex. Aycicek et al. [40] have studied the total aflatoxin levels in raw hazelnuts. According to this study, only one of 51 raw hazelnut samples has been reported to exceed this legal limit. Especially in raw hazelnut samples, high moisture content and therefore aflatoxin content were detected in high rainfall areas, indicating that climatic data play an important role. Aluç and Aluç [30] determined the

levels of aflatoxins in raw nuts obtained from Akçakoca, Ordu and Giresun. In 10 of 186 lots raw nuts from Akçakoca region $B_1 = 2.66-75.08$ ppb, total = 4.77-78.93 ppb, 20 of 160 lots raw nuts from Giresun region $B_1 = 2.17-75.68$ ppb, total = 4.34-150, 08 ppb, and 16 of 177 lots raw nuts from Ordu region $B_1 = 2.1-29.44$ ppb, total = 3.61-135.51 ppb aflatoxin contamination. Due to the high rainfall in the regions where raw hazelnuts are grown, factors such as high moisture values and the fact that hazelnuts are not fully developed while in the tree cause aflatoxin formation. In addition, mistakes made during the production, processing and storage stages lead to an increase in the amount of aflatoxin.

Aflatoxin was determined in 7 of the 50 roasted hazelnut samples and total amount of aflatoxin was between 0.1-4.04 ppb. The amount of aflatoxin found in the samples was found to be below the legal limit specified in the Turkish Food Codex. Heperkan [31] has investigated the mycotoxins in foods and their importance in terms of our country. In this study, 140 samples obtained from Ordu, Giresun and Tirebolu in Blacksearegion were

examined and aflatoxin was determined in 5 of 25 hazelnut samples. Deabes and Al-Habib [34] reported that *Aspergillus* species, which are capable of producing aflatoxin, were predominant in mycoflora of hazelnut samples and aflatoxin levels were found to be between 41-55 ppb.

Aflatoxin content was found to be 14.0% in roasted hazelnut samples. This value was found to be lower than in raw hazelnut. Raw nuts are more risky in terms of aflatoxin, while roasted nuts are more stable [41]. The reason for this is that some fungi in the mycoflora become ineffective and the moisture content decreases as a result of heat treatments applied to roasted hazelnuts. Because fungal contamination is more common due to the high rate of free water required for the development of fungi. In addition, as the fat content of the products increases, the rate of mold and aflatoxin increase [42].

In a study, aflatoxin levels were investigated in hazelnuts. *Aspergillus* species capable of producing aflatoxin in the mycoflora of hazelnut samples analyzed have been reported to be predominant and aflatoxin levels vary between 41-55 ppb [34].

In a study investigating natural mycoflora and aflatoxin contamination in 25 raw and 25 roasted hazelnut samples, aflatoxin contamination was reported in 33 (66%) of 50 hazelnut samples at 0.1–155 ppb levels. In this study, aflatoxin formation above the legal limit was determined at 14.2-155 ppb levels in 2 raw and 6 roasted hazelnut samples [32].

Baltacı et al. [43], have collected samples during 3 years from companies exporting raw and roasted hazelnuts. According to the results of this study,

aflatoxin levels were found to be between 0.02-78.98 ppb in 3188 samples. It was determined that 3147 of these samples had aflatoxin below the legal limits and 41 of them were well above the legal limits.

The amount of aflatoxin was found to be 100% in hazelnut inner membrane. It is noteworthy that aflatoxin levels reach a high rate of 0.7-38.2 ppb. However, since there is no limitation of aflatoxin in the inner membrane in Turkish Food Codex, our samples could not be evaluated in this respect. Hazelnut membrane is a thin and brown perisperm layer that completely encapsulates the hazelnut grain and is released as a by-product during roasting process. The antioxidant capacity of hazelnut membrane is high and it is also considered as dietary fiber [44,45]. In the Blacksearegion, hazelnut inner membrane is used for different purposes such as animal feed and soil fertilizer. The absence of a legal limitation for aflatoxin contamination in the hazelnut membrane is highly risky for the health. Therefore, it is thought that it should be controlled with legal restrictions. While studies have been conducted to determine whether the hard shell protects the hazelnut against fungal contamination and thus aflatoxin formation, there is not enough work on the inner membrane. However, in a study by Özer [46] the aflatoxin content of the membrane was examined after roasting and peeling of the hazelnuts. In addition to natural contaminated hazelnuts, hazelnuts were artificially contaminated with *Aspergillus flavus* to contain aflatoxin at levels 10 and 20 ppb. According to the data obtained from the study, roasting, manual separating, mechanical separating and membrane peeling were found to reduce aflatoxin contamination and Aflatoxin B₁ content in hazelnuts. Aflatoxin B₁ level before the first roasting process was 11.28 ppb. this value was found to be 11.11 ppb after roasting. This value decreased to 0.23 ppb after peeling of the membrane. In the membrane, Aflatoxin B₁ (12.71 ppb) was reported to be higher than hazelnut. It has been reported that aflatoxin is concentrated in the membrane and damaged hazelnuts and the average reduction in aflatoxin contamination after operations is reported to be 98%.

Aflatoxin levels of 0.2-6.02 ppb were determined in 9 of 20 hazelnut paste samples used in the study. Although the rate of aflatoxin contamination in the samples was 45%, none of them exceeded the legal limit specified in the Turkish Food Codex (max.5 ppb for aflatoxin B₁, max. 10 ppb for total

aflatoxin). Vural et al. [47] examined 180 hazelnut paste samples consumed in Istanbul in terms of aflatoxin B₁ and total aflatoxin and found that 6.59% of the samples exceeded the legally acceptable maximum level. Günşen and Büyükyörük [48] reported that there were an average of 1076.5 ± 194.4 ppb aflatoxin B₁ in 25 cocoa hazelnut paste obtained from various markets in Bursa. Yu et al., [49] reported that the majority of aflatoxins are better produced by fungi in glucose, fructose-containing media. It is thought that hazelnut, which is a good substrate for *Aspergillus* species and sugar added as an additive in hazelnut paste may have increased aflatoxin production in hazelnut paste. The rate of contamination in the hazelnut paste analysed cannot be ignored. This situation makes us think that businesses producing hazelnut paste, which is a food consumed especially by young people and children, should be more careful in hazelnut intake, operational hygiene and storage.

4. CONCLUSION

The weather in the Black Sea region is rainy and continuously the harvesting season is very humid, increasing the risk of Aflatoxins contamination. Fungal contamination and consequently aflatoxins formation can occur in hazelnuts, at pre-harvest, harvest, during post-harvest applications and also in storage. According to this study results indicate that, although the risk of aflatoxin formation is present in hazelnuts and products, the most important stages to prevent aflatoxin occurrence are harvesting and drying methods including storage.

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