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Research Article

Detection of Aflatoxins in Tomato and Pepper Pastes Sold in Market Places of Istanbul, Turkey

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Abstract

The aim of this study is to determine the amount of total aflatoxins and aflatoxin B1 (AFB1) in tomato and pepper pastes sold in local market places in Istanbul to evaluate whether aflatoxin levels were within the standards regarded as safe (<5 µg kg^{-1}). For this purpose, a total of 64 samples, including 26 tomato pastes, 15 paprika pastes, and 23 chili pastes, were analysed using two different detection methods, enzyme-linked immunoabsorbent assay (ELISA) and high-performance liquid chromatography (HPLC). By ELISA, total aflatoxins were detected in 27 samples (1-2.5 µg kg^{-1}), and AFB1 was detected at a level of 1 µg kg^{-1} in 20 samples. Furthermore, 21 of 64 samples were found to contain total aflatoxins (0.21-2.34 µg kg^{-1}), with 16 of them contaminated with AFB1 (0.22-2.34 µg kg^{-1}) by HPLC. The obtained results showed that both methods were suitable for aflatoxin determination in tomato and pepper paste samples and the samples have been proven to be within the standards considered as safe.

Keywords: Aflatoxin, Tomato Paste, Pepper Paste, ELISA, HPLC.

İstanbul Semt Pazarlarında Satılan Domates Ve Biber Salçalarında Aflatoksin Tespiti

Öz

Bu çalışmanın amacı, İstanbul'daki semt pazarlarında satılan domates ve biber salçalarında bulunan toplam aflatoksin ve aflatoksin B1 (AFB1) miktarını belirlemek ve aflatoksin düzeylerinin güvenli kabul edilen standartlar (<5 µg kg^{-1}) içinde olup olmadığını değerlendirmektir. Bu amaçla 26 adet domates salçası, 15 adet acı biber salçası ve 23 adet biber salçası olmak üzere toplam 64 numune, enzim bağlantılı immünoabsorban tahlili (ELISA) ve yüksek performanslı sıvı kromatografisi (HPLC) olmak üzere iki farklı tespit yöntemi kullanılarak analiz edildi. ELISA ile 27 örnekte (1-2.5 µg kg^{-1}) aflatoksin, 20 örnekte AFB1 (1 µg kg^{-1}) bulunduğu tespit edildi. Ayrıca, HPLC sonuclarina göre, 64 numuneden 21'inin aflatoksin (0.21-2.34 µg kg^{-1}) içerdiği ve bunların 16'sının AFB1 (0.22-2.34 µg kg^{-1}) ile kontamine olduğu bulunmuştur. Elde edilen sonuçlara göre, domates ve biber salçası örneklerinde aflatoksin tayini için her iki yöntemin de uygun olduğu görülmüş ve numunelerin güvenli kabul edilen standartlar içinde olduğu kanıtlanmıştır.

Anahtar Kelimeler: Aflatoksin, Domates Salçası, Biber Salçası, ELISA, HPLC.

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1. Introduction

Aflatoxins are one of the main groups of mycotoxins synthesised as secondary metabolites by filamentous fungi, including *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* species. Among all mycotoxins, aflatoxins are the most toxic secondary metabolites (Colak et al., 2012; Kovač et al., 2018; O'Riordan and Wilkinson, 2007). More than 20 different aflatoxins are found in nature; however, aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are particularly hazardous to humankind and other animals (Rushing and Selim, 2019; Udovicki et al., 2018). Long-term or chronic exposure to aflatoxins is known to be tumorogenic, mutagenic, teratogenic, immunosuppressive, and nephrotoxic (Güntekin, 2007). Furthermore, AFB1 is classified as group I carcinogen by the International Agency for Research on Cancer (IARC, 2002). For this reason, food control, as well as periodical detections, are of vital importance since fungal contamination leads to aflatoxins' accumulation, which can occur due to temperature and humidity conditions on feed and food during growth, processing, post-harvest operations and/or storage (Ardic et al., 2009; Aydin et al., 2007). The most notable aflatoxin contamination can be found in food and feed, such as nuts, dried fruits, cereals, spices, crude vegetable oils, cacao beans, and dairy products (Kabak and Var, 2006; Oruç, 2005; Yentür and Er, 2012). Due to the risk of contamination, tolerance levels of aflatoxin regulations have been enacted in most countries, including Turkey (Colak et al., 2012). According to the Turkish Food Codex, total aflatoxins and AFB1 amounts must not exceed 10 µg kg^{-1} and 5 µg kg^{-1}, respectively (Turkish Food Codex, 2011).

Tomato (*Solanum lycopersicum*) and pepper (*Capsicum spp*.) are usually consumed either fresh or after processing into various products and are two of the essential ingredients in most cultures' cuisines. Besides the consumption of fresh fruits, tomato and pepper pastes are important elements of the daily human diet since paste making is a widely used technique to preserve the food for future use. On the other hand, tomato and pepper consumption has been proposed to reduce the risk of several chronic diseases, such as cardiovascular diseases and certain types of cancer, because of their antioxidant content (Capanoglu et al., 2008; Zhang et al., 2015). According to 2017 statistics, Turkey is the 3rd largest tomato and pepper (including chilies) producer globally, producing 12.7 million tons of tomato and 2.6 million tons of pepper and chilies per year (FAOSTAT, 2017). Due to the large use of these products by the population, as well as all health aspects, possible presence of mycotoxins, particularly aflatoxins, are of great interest as a public health issue.

Various instrumental techniques have been developed to detect total aflatoxins and particularly AFB1 in a variety of samples, including gas chromatography (GC), high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and LC coupled with tandem mass spectrometry (LC-MS/MS). However, these methods can require precise equipment, professional technicians, and generally long hours or days to obtain data. Therefore, it is necessary to opt for the best method for specific conditions (Feng et al., 2020). Even though several aflatoxin detection techniques exist, due to strict regulations of the authorities, it is essential for food and feed producing companies to opt for a rapid, reliable, sensitive, convenient, and cost-effective techniques to detect aflatoxins while they must monitor their products regularly to ensure that aflatoxin levels are below regulatory limits. In this regard, HPLC and ELISA techniques have been used for the past two decades; however, the available information on comparing these techniques is limited (Beyene et al., 2019; Chiavaro et al., 2001, Set and Erkmen, 2010). In recent years, studies on comparative immunoaffinity and chromotography techniques to detect aflatoxin contamination, has been a topic of interest (Maggira et al., 2022)

In this study, 64 different tomato, paprika, and chili pastes collected from local Istanbul marketplaces were analysed for their total aflatoxins and AFB1 contents. This study aims to reveal the sanitary conditions of highly demanded commercial products: tomato, pepper, and chili pastes. In this context, total aflatoxins and AFB1 contents were investigated using ELISA and HPLC methods to ensure the consistency of these extensive techniques.

2. Material and Method

In this study, 64 different tomato and pepper pastes were collected from local marketplaces in Istanbul and investigated in terms of their aflatoxin and AFB1 contents via ELISA and HPLC.

2.1. Sample Collection

Research material was obtained from local market places of 12 different districts in the city of Istanbul, Turkey. Exact localities are shown in Figure 1. Briefly, 26 different tomato pastes, 15 different paprika pastes, and 23 different chili pastes were collected to be investigated for their aflatoxin and AFB1 content. As soon as the materials were collected, each sample was kept in a closed container and stored at +4°C until the time of analysis.

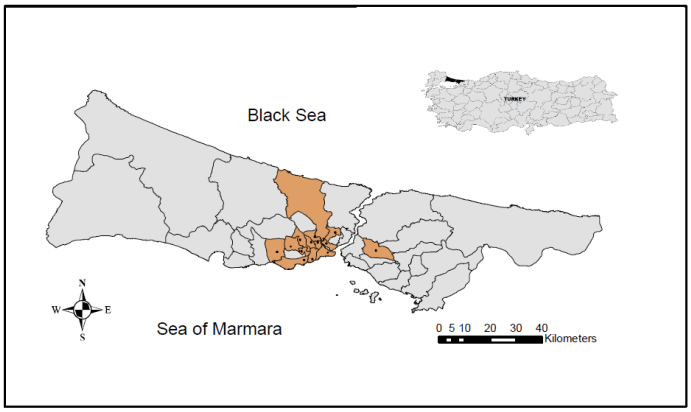


Figure 1. Sample material was collected from marked districts in Istanbul, Turkey. Şekil 1. Numuneler İstanbul'daki işaretli ilçelerden toplanmıştır.

2.2. Sample preparation and determination of total aflatoxins and AFB1 by ELISA

Each sample was weighed as 10 g and dissolved in 50 mL 80% acetonitrile at a ratio of 1:5 (w/v). The samples were shaken vigorously at 300 rpm for 5 min using a laboratory shaker. Homogenous extracts were filtered through a filter paper (Whatman N°1). Then, an aliquot of the filtrate was diluted in a ratio of 1:10 with a wash buffer, which is phosphate-buffered saline (PBS).

Each sample was analysed using a commercial kit, namely, Helica Low Matrix Total Aflatoxin and Aflatoxin B1 Kits (Helica Biosystems, Inc., Santa Ana, CA, USA) according to the manufacturer's protocol. The resulting color's optical density (OD) was measured at 450 nm by an ELISA plate reader (BioTek, Power Wave XS2 ELISA plate reader). Standards without any aflatoxin were defined as 100% maximum binding value. The remaining standards (0.02, 0.5, 0.1, 0.2, and 0.4 ng mL^{-1}) and the samples' mean values were defined according to Equation 1. All the samples were analysed in duplicate.

$$\frac{Standard\ or\ sample\ absorbance\ value}{Maximum\ binding\ absorbance\ value} \times 100 = \frac{\%B}{B0} \quad (1)$$

2.3. Sample preparation and determination of total aflatoxin and AFB1 by HPLC

HPLC analysis of samples was carried out according to the standard AOAC 999.07 method. Stock solutions of the standards (Supelco, Inc) containing 1000 ng mL^{-1} AFB1, 200 ng mL^{-1} AFB2, 1000 ng mL^{-1} AFG1, and 200 ng mL^{-1} AFG2 were dissolved in 98:2 toluene-acetonitrile (v/v) solution. Working solutions were diluted from these stocks freshly according to the method of Stroka et al. (2000).

To prepare the samples for HPLC, each 50 g of the paste sample was mixed with 5 g NaCl and dissolved in 300 mL methanol:water (80:20, v/v) solution. The solution was homogenised by blending at 22000 rpm for 3 minutes. Subsequently, extracts were cleared by filter paper (Whatman No. 1). From each sample, 10 mL of the extract was taken and diluted in 60 mL PBS, and filtered through an immunoaffinity column (Vicam AflaTest). The flow rate was adjusted to approximately 3 drops/second. Afterward, 15 mL of ultra pure water was passed through the column for washing; the air was drawn until dry. After the wash, aflatoxins were firstly eluted by passing 0.5 mL and then 0.75 mL of methanol through the column, 1 minute apart. Finally, the eluate was diluted with 1.75 mL of HPLC water and vortexed well to homogenise. A 100 µL of the aliquote was injected into the HPLC system (Agilent 1100, Agilent Technologies). Detector excitation and emission wavelengths were fixed to 360 nm and 430 nm, respectively. The eluate was passed through a C18 column (Supelco 250 mm × 4.6 mm, 5 µm particle size). The mobile phase was composed of water:acetonitrile:methanol in the ratio of 6:2:3 (v/v/v). The flow rate was adjusted to 1 ml min^{-1} and the current to 100 µA. All the samples were analysed in duplicate. Quantification of each aflatoxin was obtained by calculating peak areas at their retention times and comparing them with their relevant standard calibration curve.

2.4. Statistical analysis

Deviations between the two methods were shown with a confidence interval of 95% ($p<0.05$). The standard results of ELISA and HPLC were compared with each other using correlation analysis.

3. Results and Discussion

In the present study, total aflatoxins and AFB1 contents of 64 different tomato and pepper paste samples were evaluated using ELISA and HPLC. Statistically, a comparison of the quantitative analysis of total aflatoxin standards by ELISA and HPLC exhibited a good correlation with a correlation coefficient value of >0.96.

Limit of detections (LOD) for aflatoxins B1, B2, G1 and G2 were 0.2, 0.1, 0.3, 0.5 ng mL^{-1}, respectively by HPLC. These results were calculated with empirical methods. The chromatogram in Figure 2 demonstrates the standard retention times (min): 8.966, 10.242, 12.135, 14.009 for AFG2, AFG1, AFB2 and AFB1, respectively.

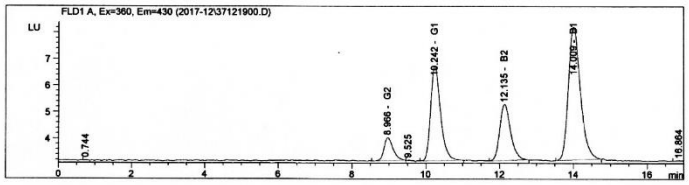


Figure 2. HPLC chromatogram indicates standard peaks of AFG2, AFG1, AFB2 and AFB1. **Şekil 2.** HPLC kromotogramı aflatoksin AFG2, AFG_1, AFB2 ve AFB1 standart piklerini gösterir.

Recovery percentages ranged from 82.71 to 105.57%, with a mean value 98.62% and relative standard deviation (RSD) value for ELISA was 9.2% (Table 1). For HPLC, the recovery was between 94.35 and 117.12%, with an average of 102.43%. On the other hand, RSD value of HPLC was found to be 8.69% (Table 1). These results coincide with the previously announced criteria of RSD ≤ 15%, which regarded as a good precision of the methods Omar et al. (2020). The mean recovery rate of HPLC was higher and more stable than the percent recovery rate obtained from ELISA. However, there was no significant difference found in the

Table 1. Recovery rates of aflatoxins in standards by ELISA and HPLC methods. Tablo 1. Aflatoksin standartlarının ELISA ve HPLC metotlarından elde edilen geri kazanım oranları

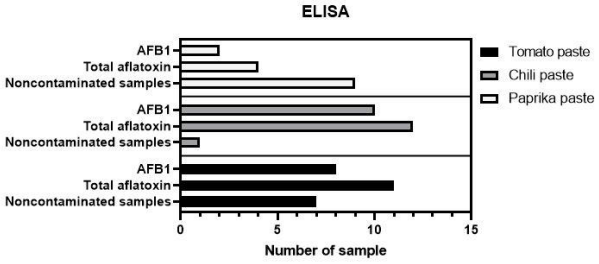
ELISA		HPLC	
Standard (ng mL^{-1})	**Recovery (%)**	**Standard (ng mL^{-1})**	**Recovery (%)**
0.02	99.66	0.960	117.12
0.05	101.04	2.880	99.51
0.1	105.57	4.800	94.35
0.2	104.12	6.720	98.93
0.4	82.71	8.640	102.24

recovery rate between both methods. This result was also reported by Beyene et al. (2019).

Our data revealed that, total aflatoxins were identified in 11 tomato, 12 chili and 4 of the paprika paste samples (42.2% of all samples) according to ELISA results ranged from 1 to 2.5 µg kg^{-1}. Furthermore, of the total aflatoxin-contaminated products, 8 tomato, 10 chili, and 2 paprika paste samples (74.1% of total aflatoxin-contaminated samples) were proven to be contaminated with AFB1 at the level of 1 µg kg^{-1} (Figure 3 a, Table 2).

On the other hand, HPLC results revealed that 7 tomato (0.21-1.66 µg kg^{-1}), 11 chili (0.22-2.18 µg kg^{-1}) and 3 of the paprika (0.35-2.34 µg kg^{-1}) paste samples (32.8% of all samples) were contaminated with total aflatoxins in a range of 0.21 to 2.34 µg kg^{-1}. Among these, 6 tomato (1.27-0.33 µg kg^{-1}), 8 chili (0.22-1.14 µg kg^{-1}), and 2 paprika paste (0.68-2.34 µg kg^{-1}) samples (59.3% of total aflatoxin-contaminated samples) were proven to be contaminated with AFB1 at a level ranged between 0.22-2.34 µg kg^{-1} (Figure 3 b, Table 2). As such, no samples exceeded the maximum limit of the Turkish Food Codex (<5 µg kg^{-1}).

a.



b.

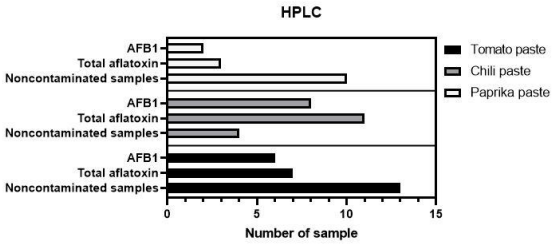


Figure 3. a. Number of noncontaminated samples and contaminated samples containing total aflatoxin and AFB1 according to ELISA results. b. Number of noncontaminated samples and contaminated samples containing total aflatoxins and AFB1 according to HPLC results. Şekil 3. a. ELISA sonuçlarına göre toplam aflatoksin ve AFB1 içeren kontamine örnek ve kontamine olmayan örnek sayısı. b. HPLC sonuçlarına göre toplam aflatoksin ve AFB1 içeren kontamine örnek ve kontamine olmayan örnek sayısı.

Total aflatoxin and AFB1 detection by ELISA and HPLC were compared in Table 2. A substantial part of both results were consistent in terms of total aflatoxins and AFB1. However, there were also some differences, such as in samples 18, 33, 34, 35, 64, and 72, where 1 µg kg^{-1} of total aflatoxins was detected by ELISA; on the contrary, no aflatoxins were detected by HPLC. Similarly, sample numbers 36, 48, 65, and 72 were detected to be AFB1 positive by ELISA but failed by HPLC. Moreover, our data show that ELISA assay results were mostly higher than HPLC, which was also stated by Colak et al. (2006). This disparity in the results could be caused by limitations of the ELISA assay. As also stated by Rossi et al. (2012), immunoassays such as ELISA can show inflated results since the sample matrix can contain similar epitopes, resulting in unspecific antibody binding.

Even though ELISA offers many advantages, for instance, a short analysis time, simple sample preparation, and low cost, suspicious or irreproducible results must be confirmed with additional and more accurate techniques (e.g., HPLC). (Kos et al. 2016).

Mariutti and Soares (2009) also evaluated the existence of aflatoxins in different tomato-based products, including ketchup, pulp paste, and puree. According to their results, all aflatoxin levels of the samples were found to be within recommended limits. Similarly, in Italy, Mutti et al. (1992) detected 70 commercially available tomato products and none of them were contaminated with aflatoxins more than 1 µg kg^{-1}. To our knowledge, there are only a few studies addressing aflatoxin investigation in tomato products via ELISA and HPLC. In this regard, our study will serve to extend the up to date literature to this context.

Furthermore, Yentür et al. (2012) has inspected 90 different pepper pastes which were collected from supermarkets in Ankara and analysed for AFB1 by ELISA. According to the results of this study, 69 of 90 samples were detected to contain AFB1 lower than 1.25 µg kg^{-1}, 16 samples were between 1.25-2.00 µg kg^{-1}, and only 5 of the samples were contaminated at a relatively higher level varying from 2.00 to 4.00 µg kg^{-1}. Similarly, none of the samples exceeded the level of 5 µg kg^{-1}. In contrast, Aydin et al. (2007) examined 100 powdered red pepper samples collected from Istanbul markets and found that 18 of 100 samples were contaminated with AFB1 above the maximum limit (>5 µg kg^{-1}). Gambacorta et al. (2017) reported that 31% of 45 pepper samples collected from a variety of cities in Italy were found to be aflatoxin-contaminated, and 2 of the samples were above the European Union limit (<5 µg kg^{-1}) with a result up to 12.8 µg kg^{-1} for AFB1. According to Garduño-García et al. (2017), 95% (51/54) of pepper samples were found to be contaminated with AFB1, and only 9.26% of the samples were under the Mexican legislation limit. In another study carried out in Pakistan by Iqbal et al. (2017), total aflatoxin contamination of 312 chili samples, including crushed chili, chili powder, chili sauce, and whole chili samples were evaluated: 56.4% (176/312) of the samples were positive for aflatoxins. Acaroz (2019) found that, in Afyonkarahisar, Turkey, 49 of the 76 pepper samples (64.47%) contained aflatoxins (1.76-42.72 µg kg^{-1}) and 5 of the samples (6.58%) exceeded the regulatory limits in Turkey and the European Union. These results show that aflatoxins are a worldwide concern in agriculture, food processing, and human health. This regard demonstrates the importance of regular monitoring of aflatoxin content in food products.

Table 2. Average amount of total aflatoxins and AFB1 in positive samples. Tablo 2. Pozitif numunelerdeki ortalama aflatoksin ve AFB1 miktarları

		ELISA		HPLC	
Sample Nr.	**Product Type**	**Total aflatoxin ($\mu g\ kg^{-1}$)**	**Aflatoxin B1 ($\mu g\ kg^{-1}$)**	**Total aflatoxin ($\mu g\ kg^{-1}$)**	**Aflatoxin B1 ($\mu g\ kg^{-1}$)**
17	Tomato paste	1	1	1.66	0.9
18	Tomato paste	1	<LOD	<LOD	<LOD
24	Tomato paste	1-2.5	1	0.33	0.33
33	Tomato paste	1	<LOD	<LOD	<LOD
34	Tomato paste	1	<LOD	<LOD	<LOD
47	Tomato paste	1	1	1.27	1.27
53	Tomato paste	1	1	0.61	0.61
65	Tomato paste	1	1	0.21	<LOD
69	Tomato paste	1	1	0.64	0.64
72	Tomato paste	1	1	<LOD	<LOD
78	Tomato paste	1	1	0.93	0.93
25	Chili paste	1	1	0.98	0.98
35	Chili paste	1	<LOD	<LOD	<LOD
36	Chili paste	1	1	0.56	<LOD
39	Chili paste	1	1	0.84	0.84
44	Chili paste	1	1	0.22	0.22
48	Chili paste	1-2.5	1	2.18	<LOD
51	Chili paste	1	<LOD	0.8	<LOD
54	Chili paste	1	1	0.65	0.65
66	Chili paste	1	1	1.14	1.14
79	Chili paste	1	1	0.54	0.54
81	Chili paste	1	1	0.78	0.78
84	Chili paste	1-2.5	1	0.59	0.59
61	Paprika paste	1-2.5	1	2.34	2.34
64	Paprika paste	1	<LOD	<LOD	<LOD
26	Paprika paste	1	<LOD	0.35	<LOD
67	Paprika paste	1	1	0.92	0.68

4. Conclusions and Recommendations

In summary, by both ELISA and HPLC, none of the samples' aflatoxin concentrations exceeded the limit of the Turkish Food Codex (maximum 5 $\mu g\ kg^{-1}$). This outcome is quite important to ensure the safety of consumers. Since even a small amount of mycotoxin-contaminated food is known to cause carcinogenic effects with long-term consumption, aflatoxin-contaminated food poses a high risk to human health. Therefore, their regular detection in products of high demand is crucial. Moreover, this research demonstrates the validation of ELISA and HPLC for the detection and quantification of aflatoxins in tomato and pepper pastes available in the marketplaces in Turkey. It was found that, both ELISA and HPLC are appropriate techniques for aflatoxin detection since these two approaches are strongly correlated with each other. The selection of analytical methods mostly depends on the availability, cost, and the number of samples. ELISA has some advantages, such as rapid testing, lower cost, and simplicity however, HPLC is more accurate and specific compared to ELISA.

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