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Occurrence and dietary risk assessment of AFB1 in tomato and pepper pastes in Türkiye

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ORIGINAL ARTICLE

Abstract

Tomato and pepper pastes are essential ingredients in Mediterranean and Middle Eastern cuisines and contain beneficial health components. However, they may also contain toxic substances, such as aflatoxins. This study aimed to measure the levels of aflatoxin B1 (AFB1) in commercially branded and locally produced tomato and pepper pastes, and to determine the association between water activity (AW) and AFB1 contamination. Additionally, a risk assessment for AFB1 was conducted by calculating the estimated daily intake (EDI), margin of exposure (MOE), and hepatocellular carcinoma (HCC) risk. An evaluation of paste type (tomato or pepper) and source (branded or unlabeled) showed no statistically significant difference in AFB1 contamination ($p>0.05$). No statistically significant correlation was discovered between AW and AFB1 contamination in samples of tomato and pepper pastes ($p>0.05$). Evaluation of the data revealed that although the observed AFB1 levels were quite low, EDI, MOE, and HCC values were high. This may be due to the high daily consumption of tomato paste. Therefore, public health authorities must prevent AFB1 contamination in foods having high daily consumption. Legal limits of AFB1 contamination in such foods should be reduced as much as possible or not allowed at all.

Keywords: aflatoxin B1; dietary exposure; dietary risk assessment; tomato and pepper pastes; water activity (AW)

Introduction

Today, consumers are very sensitive about food safety and food contaminants. Mycotoxins, among the most significant food contaminants, negatively impact public health, food safety, and the national economies of many countries, particularly developing nations (Heshmati and Khorshidi, 2021). Fungi, one of the main causes of productivity loss in agricultural production, contaminate foods before, during, and after harvest. Damage from mycotoxin-producing fungi (which produce secondary metabolites) extends beyond fruit, seriously compromising the quality of processed products and posing risks to food safety (Bryden, 2012; Lee *et al.*, 2015; Santos *et al.*, 2016). Aflatoxins are the most toxic secondary metabolites of all mycotoxins (Bryden, 2012; Fang *et al.*, 2022; Pisoschi*et al.*, 2023). Aflatoxins, which are produced via the polyketide pathway by various species of *Aspergillus flavi*, especially *A. flavus* and *A. parasiticus*, and have a chemically difuranocoumarin structure (Winter and Pereg, 2019), are highly toxic secondary metabolites (Kim *et al.*, 2019; Udomkun *et al.*, 2017).

Although more than 20 different aflatoxins are present in nature, aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) are the most dangerous types, especially for humans and animals. Prolonged or chronic exposure to aflatoxins is recognized to induce

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tumorigenic, mutagenic, teratogenic, immunosuppressive, and nephrotoxic effects. Among these, AFB1 is recognized as the most carcinogenic aflatoxin for both humans and animals (Fang *et al.,* 2022; Safavizadeh *et al.,* 2022). Therefore, the International Agency for Research on Cancer (IARC, 2012) has classified AFB1 as a group 1 carcinogen.

Numerous studies have suggested that the production of mycotoxins, such as AFB1, is primarily linked to environmental conditions (Gizachew *et al.,* 2019; Mannaa and Kim, 2017). Abiotic factors, such as temperature, water activity (AW), and their interactions, are reported as key factors modulating fungal growth and the production of secondary metabolites (Al-Zaban, 2023; Liu *et al.,* 2017; Medina *et al.,* 2017; Schmidt-Heydt *et al.,* 2010). Thermal techniques, such as roasting and baking, and nonthermal applications, such as irradiation, grinding, and fermentation processes applied to foods, can cause a decrease in aflatoxin levels, but cannot completely eliminate aflatoxins in processed food products (Kabak, 2021). In the European Union (EU) and Türkiye, the legal limit for AFB1 is accepted as 5 μg/kg (European Commission, 2010; Turkish Food Codex [TFC], 2011).

The Rapid Alert System for Food and Feed (RASFF, 2023) recorded 6729 notifications in all categories from January 2020 to June 2023. Of these, 570 (8.47%) were related to mycotoxins and 429 (6.37%) were specifically about aflatoxins. In RASFF, 113 (26.34%) of aflatoxin warnings were for foods originating from Türkiye. Two notifications about 'tomato paste' were identified in tomato paste originating from Italy, one of which contained Alternaria toxins and the other contained mycotoxins. However, no notifications are identified related to pepper paste in RASFF (2023).

Aflatoxin is a significant environmental toxin that plays a role in the development of HCC, particularly in the regions having high contamination of dietary foodstuffs, such as peanuts, corn, Brazil nuts, pistachios, spices, and figs. AFB1 is awell-known aflatoxin that causes mutations leading to cancer development because of its genotoxic properties (Akyerli *et al.,* 2020; Mizrak *et al.,* 2009). Joint Expert Committee on Food Additives (JECFA) and the European Union Scientific Committee on Food have warned that even very low levels of exposure to total aflatoxins (AFTs) (<1 ng/kgbody weight [bw]/day) may increase the risk of liver cancer. Owing to aflatoxins being genotoxic carcinogens, their levels within food items should be regularly monitored and reduced by adhering to the, as low as reasonably achievable (ALARA), principle of keeping minimum exposure to aflatoxins (European Food Safety Authority [EFSA], 2007).

The Food and Agriculture Organization–World Health Organization (FAO-WHO) Joint Expert Committee on Food Additives (JECFA) evaluated aflatoxins in 1987, 1997, and 2007. Owing to their genotoxic and carcinogenic properties, no observed adverse effect level (NOAEL) or tolerable daily intake (TDI) was specified (EFSA, 2007; Oktay Basegmez, 2019). Therefore, the limit of exposure and cancer potency estimates based on epidemiological and toxicological studies are used for risk characterization (Oktay Basegmez, 2019).

Given this, it is vital to evaluate dietary exposure through the regulation of mycotoxin levels in food items and consideration of consumer consumption patterns (Şen and Civil, 2022). Risk assessment, which provides scientific guidance on food-related risks, consists of the following four steps: (a) hazard identification, (b) hazard characterization, (c) exposure assessment, and (d) risk characterization (EFSA, 2012). In Türkiye, although AFB1-induced risk assessments were conducted in hazelnut (Şen and Civil, 2022), almonds (Kanik and Kabak, 2019), figs (Oktay Basegmez, 2019), chocolate products and peppers (Kabak, 2019, 2021; Özlü, 2024), no studies, to our knowledge, are conducted on AFB1 exposure in tomato and pepper pastes consumption.

Tomato (*Solanum lycopersicum*) and red pepper (*Capsicum spp.*) are fundamental components of human diet, particularly in Mediterranean cuisine. They are commonly consumed as fresh or processed invarious products for longer preservation. One of the most significantly derived products is tomato paste, which enhances the taste, smell, aroma, and appearance of dishes (Chaudhary *et al.,* 2018; Öner*et al.,* 2022; Tagliamonte *et al.,* 2023). Türkiye is one of the important countries in tomato and red pepper production because of its favorable climatic conditions. While these products are primarily cultivated in China, Türkiye is among the top five global producers of tomato and red pepper. Türkiye is also one of the leading producers of tomato paste, ranking fourth after the United States, China, and Italy (FAOSTAT, 2022).

According to the Turkish Food Codex, tomato paste is defined as a product made by removing the skin, core, and fiber from ripe, firm, and red tomatoes by chopping them. The tomato pulp is then thickened to a minimum of 28% brix without adding any extra salt, and preserved through physical methods. Pepper paste, on the other hand, is made by thoroughly washing and crushing fresh, ripe, and firm red peppers, both hot or sweet varieties. The peppers are heated and either the skin, core, and fibers are removed or left intact, depending on the desired method. The pepper pulp is then thickened to a minimum of 18% brix without any added salt, and preserved through physical means (TFC, 2020).

Although pepper paste is mainly produced using traditional methods, tomato paste is typically produced through industrial processes. In industrial production, paste is usually packed in hermetic containers, while traditional methods often use polyethylene packaging. The salt content in tomato paste must not exceed 5%, while traditional paste can have a higher salt level. As a result, pastes made through traditional methods have a lower AW and higher dry matter content, which help to enhance the product's shelf life (Ayda *et al*., 2023).

According to 2021 statistics, 538.405 tons of tomato paste and 60.540 tons of pepper paste are consumed annually in Türkiye (Anonymous, 2021). Owing to the widespread use of these products by the society and considering their health aspects, the possible presence of mycotoxins, especially aflatoxins, is of great interest as a public health problem.

In Türkiye, incidences of high aflatoxinsin tomato and pepper pastes couldbe a concern because these are consumed extensively on daily basis. For this reason, these two food products were specifically selected for exposure assessment and risk characterization. This study provides the first description of aflatoxin contamination in tomato and pepper pastes marketed in Türkiye. No study investigating the potential cancer risk caused by AFB1 contamination through tomato and pepper pastes has been conducted in our region or globally. The aims of this study were (a) to determine the levels of AFB1 contamination and AW in branded and unlabelled tomato paste consumed in Türkiye; (b) to analyze the relationship between these two sets of data; and (c) to estimate dietary exposure and conduct risk characterization through the consumption of tomato and pepper pastes in Türkiye, highlighting the potential public health risks associated with AFB1.

Materials and Methods

Sample collection

Tomato paste and pepper paste samples were either branded or sourced locally (unlabeled). Samples (N=160; 50 samples of branded tomato paste, 50 of unlabelled tomato paste, 30 of branded pepper paste, and 30 of unlabelled pepper paste) were collected randomly from retail shops, local markets, and bazaars in different provinces of Türkiye, such as Erzurum, İstanbul, Adana, Şanlıurfa, Gaziantep, Ankara, İzmir, and Hatay from September 2022 until February 2023. The samples were transported to the laboratory under a cold chain and stored in a refrigerator (4°C) for analysis. In the study, the collected branded tomato paste samples were produced industrially and sold in hermetically sealed tin or glass containers. The unlabeled samples were made using traditional methods and often sold in polyethylene packaging.

Measuring the AFB1 and AW

An enzyme-linked immunosorbent serologic assay (ELISA) was employed to determine the AFB1 levels in tomato paste and pepper paste samples, using the RIDASCREEN® Aflatoxin B1 test kit (Aflatoxin B1 30/15, Art. No.: R1211; R-Biopharm AG, Darmstadt, Germany). This method includes a single-use immunoaffinity RIDA® Aflatoxin column (R-Biopharm AG) for sample clean-up before the analysis of aflatoxin B1. The columns are particularly suited for the cleanup of difficult samples, such as nuts, herbs, spices, and tea leaves (R-biopharm AG). At room temperature, each immunoaffinity column was filled with 1 mL of previously prepared sample solution. The sample was passed slowly and continuously through the column at a flow rate of approximately 1 drop/s to prevent compression of the gel and thus possible loss of aflatoxin. After the permeated solution was discarded, the column was rinsed with 10 mL of distilled water and the permeated solution was discarded again. Some air was introduced into the column to ensure that all remaining liquid was removed from the column. The syringe was then removed and a clean, closable vial was placed directly under the column. To ensure complete elution of aflatoxins, 0.5 mL of pure methanol was slowly passed through the column. This step was repeated when the eluent passed too quickly. All traces of eluent were collected by thoroughly pushing air through the column.

Purification of the extract with immunoaffinity columns increased specificity and sensitivity, resulting in increased accuracy and sensitivity. Pure extracts were obtained based on the antigen–antibody reaction. The column contained a gel suspension to which monoclonal antibodies were bound covalently. Antibodies were specific for aflatoxin B1, B2, G1, and G2. As the aflatoxins in the sample passed through the column, they were bound to monoclonal antibodies, while all other substances were removed (Macri *et al.,* 2020).

The cleanup procedure was followed by discovering of AFB1. For this purpose, 50 µL of toxin-containing eluent (sample resulting after the cleanup process) was diluted with 450 µL of distilled water. The test was performed byadopting manufacturer's instructions. The test kit included AFB1 standards (encompassing 0, 5, 10, 20, 40, and 80 µg/kg). Any sample with AFB1 levels below the minimum detection limit of the assay was classified as negative for AFB1,

$$\text{Limit of detection (LOD)} = 1\ \mu g/kg$$

The AW was measured by utilizing Aqualab 4TE AW meter (Aqualab 4TE; Decagon Devices, Pullman, WA, USA). In our study, water activity values were determined in the range of 0.7-0.99 in all tomato paste samples. In light of these data, better interpret the relationship between AW and AFB1 in both tomato and pepper paste samples, the AW values were divided into the following three groups: group 1 (0.70–0.79), group 2 (0.80–0.89), and group 3 (0.90–0.99).

Estimated daily intake (EDI)

Since mycotoxin formation data in foods and food consumption data are evaluated together to estimate dietary exposure, mycotoxin formation data mustbe evaluated accordingly. The most preferred methods when making dietary evaluations are the use of lower bound (limit) (LB) and upper bound (limit) (UB) values. However, according to the European Food Safety Authority (EFSA), to give more accurate results, left-skewed data mustbe handled by the substitution method, taking into account the percentage of left-censored valuesin all data (up to 60% of uncensored data). When samples contain a high number of left-censored data, the general approach is as follows: (1) assign zero value for LB estimate, (2) assign LOD/2 or limit of quantification (LOQ/2) for middle bound (limit) (MB) estimate, and (3) LOD for UB estimate, or assign LOQ (EFSA, 2010). Since the data in this study were skewed to the left, the substitution method was used.

Total EDI values of AFB1 (ng/kg bw/day) were computed using Equation (1) (Calderón *et al.,* 2023):

$$\text{Total EDI} = \text{Di} \times \frac{\text{M}}{\text{W}}, \quad (1)$$

where Di represents the daily consumption (g/person/day) of paste sourced from Türkiye (Anonymous, 2021); Mi represents the average AFB1 concentration, measured in ng/g, while W represents the body weight in kilograms (kg). When calculating dietary exposure of adults to AFB1, a body weight of 73.7 kg was employed, as recommended by the national ınstitute of statistics, that is, Turkish Statistical Institute (TUIK, 2023).

Dietary exposure estimates were calculated for AFB1 based on both mean (LB, UB, and MB) and 95th percentile.

Health risk characterization

Margin of exposure (MOE) and cancer potency estimates were used to determine the health risk arising from the aflatoxin content of consumed tomato paste. When calculating the MOE for AFB1, the 95% LB on the benchmark dose corresponding to a 10% extra risk ($BMDL_{10}$) value of 0.4 μg/kg bw/day, which is considered the most appropriate study result by EFSA (2020), was used. MOE ≥ 10,000 is considered a value of low risk to public health (Bouelet Ntsama *et al.,* 2023, Ezekiel *et al.,* 2021; Udovicki *et al.,* 2021; Wang *et al.,* 2018).

The MOE value is calculated using Equation (2) given below:

$$\text{MOE} = \frac{\text{BMDL10}}{\text{EDI}} \quad (2)$$

The risk of liver cancer inthe Turkish population was evaluated according to EDI results, and the average carcinogenic potency factor (Pcancer) was calculated using the prevalence of chronic hepatitis. The carcinogenic potency factor of AFB1 was further calculated considering the prevalence of hepatitis B virus surface antigen (HBsAg) positive individuals in aparticular population (Udovicki *et al.,* 2021). For the Turkish population, $HBsAg^{+}$ value of 4% was used, which is the rate reported in arecent study conducted by Özkan, (2018) in Türkiye. The risk of AFB1-related liver cancer was calculated by the product of EDI and Pcancer (Equation 3),

$$\text{Pcancer} = 0.01 \times \text{HBsAg}^{-}\,(\%) + 0.3 \times \text{HBsAg}^{+}(\%) \quad (3)$$

$$\text{Pcancer} = 0.01 \times 0.96 + 0.3 \times 0.04 = 0.022.$$

where Pcancer is the target population liver cancer risk; $HBsAg^{+}$ is the population fraction of surface antigen-positive cases of hepatitis B virus; and $HBsAg^{-}$ is the population fraction of surface antigen-negative cases of hepatitis B virus.

Based on this carcinogenic potency, the annual risk of HCC incidence was calculated as follows (Equation 4):

$$\text{HCC} = \text{EDI} \times \text{Pcancer} \quad (4)$$

Statistical analyses

The data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's comparison test using SPSS 19.0 (IBM, Chicago, IL, USA). The data were presented as mean ± standard error (SE), percentage distribution, and frequency numbers. Values were regarded as significantly different at $p < 0.05$.

Results and Discussion

AFB1 and AW findings

AFB1 and AW values of branded and unlabelled tomato paste and pepper paste samples are presented in Table 1.

Table 1. Comparison of AFB1 and AW values of unlabelled and branded tomato paste and pepper paste samples.

Paste type	N	AW			AFB1(μg/kg)			Positive (n/%)*	Above the legal limit**
		Mean±SE	Min.	Max.	Mean±SE	Min.	Max.		
Unlabelled tomato paste	50	0.8971±0.0111[b]	0.7516	0.9776	1.47±0.16	1.11	1.82	4/8	ND
Branded tomato paste	50	0.9209±0.0074[a]	0.8293	0.9710	1.75±0.19	1.57	1.194	2/4	ND-
Total tomato paste	100	0.9083±0.0070[A]	0.7516	0.9776	1.56±0.13	1.11	1.94	6/6	ND
Unlabelled pepper paste	30	0.8414±0.0125[b]	0.7025	0.9625	1.68±0.33	1.04	2.87	5/16.7	ND
Branded pepper paste	30	0.9032±0.0091[a]	0.7516	0.9592	1.71±0.39	1.03	2.82	4/13.1	ND
Total pepper paste	60	0.8723±0.0087[B]	0.7025	0.9625	1.70±0.23	1.03	2.87	9/18	ND
Total	160	0.8909±0.0057	0.7025	0.9776	1.64±0.15	1.03	2.87	15/9.4	ND

[a,b]Significant differences were observed between brands at *p*≤0.001.
[A,B]Significant differences were observed between paste types at *p*≤0.001.
*A sample was considered negative if its AFB1 concentration did not exceed 1 μg/kg, which was the detection limit of the RIDASCREEN® Aflatoxin B1 test kit.
**A sample was considered to be above the EU/Türkiye legal limit if its AFB1 concentration exceeded 5 μg/kg for tomato and pepper paste.
AW: water activity; AFB1: aflatoxin B1; N: number of samples; SE: standard error; ND: not detected.

The average AW levels of unlabeled tomato and pepper pastes were statistically lower than those of commercial tomato paste samples (*p*≤0.001). Additionally, the average AW values of tomato paste were higher than that of pepper paste. No statistical difference was observed between tomato and pepper paste samples and branded and unlabelled samples regarding AFB1 contamination (*p*>0.05). The rate of positive AFB1 contamination was 8% in unlabelled tomato paste, 4% in branded tomato paste sample, and 6% was the overall proportion. The positivity rate for AFB1 contamination in pepper paste was 16.7%, 13.1%, and 18% in unlabelled, branded, and total, respectively. Although 15 samples (9.4%) of the total 160 tomato paste samples analyzed were contaminated with AFB1, no sample exceeded the legal limit of contamination (5 μg/kg) set by the EU and Türkiye government for AFB1.

When the data of this study were examined, it was concluded that proportions and levels of AFB1 contamination were relatively low, and this couldbe due to the control of development of aflatoxin in raw material to some extent. In fact, Zahra *et al.* (2022) reported that aflatoxins were not detected in fresh tomatoes or peppers. As is well known, tomatoes and peppers are perishable items and can be contaminated by microorganisms, especially during storage. Therefore, chemical fungicides are mostly used to reduce the growth of phytopathogenic fungi in tomatoes and peppers (Segura-Palacios *et al.*, 2021). It is preferred that the peppers and tomatoes used to produce tomato paste must be unspoiled in terms of product quality and technological aspects. This is because disruption of tissue integrity in ripe tomatoes and peppers leads to the rapid breakdown of pectin by pectin-degrading enzymes. In such cases, producers donot prefer to use spoiled raw tomatoes and peppers, as consistency problems occur in the final product. Another important aspect of production of mycotoxin in foodstuffs is the presence or absence of compounds that inhibit toxin synthesis. Such compounds must be present in sufficient concentration to be partially or fully effective. Tomatoes contain polyphenols, which are able to suppress the synthesis of such toxins. However, the adequate inhibitory concentration of these compounds remains unclear (Mariutti and Valente Soares, 2009).

According to 2017 statistics, Türkiye produced 12.7 million tons of tomatoes and 2.6 million tons of pepper and hot pepper annually, making it the third largest global producer of tomatoes and peppers (including hot peppers) (FAOSTAT, 2017). Türkiye exported 715,900 tons of tomato paste and 63,338 tons of pepper paste in 2021 (Anonymous, 2021). It is known that an effective control on contaminants, such as aflatoxin, exists in export products in the world. Since Türkiye is an important exporter in this field, necessary precautions are taken by the competent authorities regarding aflatoxin contamination. No notifications emergedin RASFF (2023) between 2020 and 2023 regarding tomato paste produced in Türkiye.

It is noteworthy that only a few studies are foundin the literature regarding the presence of aflatoxin in both paste types. Mariutti and Valente Soares (2009) reported that aflatoxins B1, B2, G1, and G2 (LOD: 2–7 μg/kg) were not detected in tomato-origin products (pulp, paste, purée, ketchup, dehydrated tomatoes, and dried tomatoes preserved in oil). Another study reporting low contamination in tomato pastes (Safavizadeh *et al.,* 2021) (LOD: 0.14 μg/kg) discovered that the average AFB1 concentration was 1.1 ± 0.02 μg/kg and six out of 30 tomato pastesamples exceeded EU legal limits.

In Türkiye, Öner *et al.* (2022) discovered AFB1 (1 μg/kg) and AFTs (1–2.5 μg/kg) in 27 and the 20 samples in their study (LOD for AFB1: 0.02 ng/mL in ELISA and LOD for aflatoxins B1, B2, G1, and G2 were 0.2, 0.1, 0.3, 0.5 ng/mL, respectively) by using ELISA and high-performance liquid chromatography (HPLC) in tomato and pepper pastes. According to HPLC results, 21 out of 64 samples were discovered to contain AFTs (0.21–2.34 μg/kg), and 16 of these tomato paste and pepper paste samples were contaminated with AFB1 (0.22–2.34 μg/kg). In addition, these researchers reported that both methods were reliable for detection of aflatoxins because findings of both techniques were compatible with each other.

Association of AW with AFB1

AFB1 levels and AW values of unlabelled and branded tomato paste and pepper paste samples are presented in Table 2.

AFB1 contents of tomato paste samples were statistically similar to AW groups (p>0.05). Although there is a direct relationship between AW and aflatoxin formation, AW is not the only effective factor. Multiple factors influence the development of molds and the build-up of aflatoxins in food and feed. These include AW, temperature, pH, atmosphere composition, substrate, species interaction, and time. Relative humidity and temperature are typically regarded as the most crucial variables during drying and storage. *A. flavus* and *A. parasiticus* species prefer to grow at a temperature of 22–35°C and an AW of 0.95–0.98 (Agriopoulou *et al.*, 2020).

Gizachew *et al.* (2019) examined the development and AFB1 formation potential of *A. flavus* and *A. parasiticus* in tomato and pepper paste seeds at different temperatures and AW conditions (temperatures of 20, 27, and 35°C, and AW of 0.82, 0.86, 0.90, 0.94, and 0.98) during a 30-day incubation period. It was reported that these two fungi could develop in Nyjer (*Guizotia abyssinica*) seeds at temperatures of 20, 27, and 35°C and AW of 0.86–0.98; however, the optimal growth conditions were noted as 27°C at AW of 0.90–0.98, or 35°C at AW of 0.90–0.94. While *A. parasiticus* could produce AFB1 under all the growth conditions examined, *A. flavus* could produce AFB1 in seeds only at a temperature of 27°C with AW of 0.90–0.98 and at 35°C with an AW of 0.90. Liu *et al.* (2017) reported that the optimum growth conditions for *A. flavus* were a temperature of 37°C and an AW of 0.98, and maximum AFB1 production was achieved at a temperature of 28°C and an AW of 0.96.

Exposure levels of Turkish consumers to AFB1

The LB, MB, UB, and 95th percentile concentrations of AFB1 in tomato paste and pepper paste samples and

Table 2. AFB1 levels and AW values of unlabelled and branded tomato paste and pepper paste samples.

Paste type	AW	N	AW			AFB1 (μg/kg)		
			Mean±SE	Min.	Max.	Mean±SE	Min.	Max.
Unlabelled tomato paste	0.70–0.79	5	0.7731±0.0081	0.7516	0.7954	1.31±0.00	1.31	1.31
	0.80–0.89	18	0.8674±0.0064	0.8425	0.8963	–	–	–
	0.90–0.99	27	0.9415±0.0052	0.9028	0.9776	1.52±0.21	1.11	1.82
	Total	50	0.8971±0.0111	0.7516	0.9776	1.47±0.16	1.11	1.82
Branded tomato paste	0.70–0.79	0	–	–	–	–	–	–
	0.80–0.89	12	0.8708±0.0076	0.8293	0.8968	–	–	–
	0.90–0.99	38	0.9424±0.0050	0.9013	0.9710	1.75±0.19	1.57	1.194
	Total	50	0.9209±0.0074	0.8293	0.9710	1.75±0.19	1.57	1.194
Unlabelled pepper paste	0.70–0.79	9	0.7572±0.0135	0.7025	0.7955	–	–	–
	0.80–0.89	15	0.8578±0.0059	0.8203	0.8968	1.10±0.06	1.04	1.15
	0.90–0.99	6	0.9268±0.0110	0.9031	0.9625	2.07±0.41	1.55	2.87
	Total	30	0.8414±0.0125	0.7025	0.9625	1.68±0.33	1.04	2.87
Branded pepper paste	0.70–0.79	1	0.7516±0.00	0.7516	0.7516	–	–	–
	0.80–0.89	13	0.8712±0.00085	0.8129	0.8963	1.26±0.23	1.03	1.48
	0.90–0.99	16	0.9387±0.0043	0.9061	0.9592	2.17±0.65	1.52	2.82
	Total	30	0.9032±0.0091	0.7516	0.9592	1.71±0.39	1.03	2.82

SE: standard error; N: number of samples; AFB1: aflatoxin B1; AW: water activity.

chronic exposure estimates calculated from daily consumption patterns of tomato and pepper pastes per capita in Türkiye are summarized in Table 3.

In the study, EDI values resulting from AFB1 exposure because of tomato and pepper pastes consumption in the Turkish population were between 0.0135 ng/kg bw per day and 0.2588 ng/kg bw per day. Furthermore, the mean 95th percentile dietary exposure to AFB1 via tomato paste and pepper paste were 0.4344 ng/kg bw per day and 0.0756 ng/kg bw per day, respectively, for the Turkish population.

In literature, no EDI data related to consumption of tomato and pepper pastes was discovered. However, Kabak (2021) reported the average EDI values for AFB1 and AFT as 0.044 ng/kg bw per day and 0.047 ng/kg bw per day, respectively, while Oztekin and Karbancioglu-Guler (2022) determined the same values as 0.174 ng/kg bw per day and 0.187 ng/kg bw per day, respectively. In another study, the average EDI values of AFB1 and AFT in red pepper-containing food products for the Turkish population were reported as 0.0176 ng/kg bw per day and 0.0182 ng/kg bw per day, respectively (Özlü, 2024). Adugna *et al.* (2022) reported that the EDI values for AFB1, AFG1, AFB2, and AFG2 in red pepper ranged from 0.00064–0.015800, 0.00043–0.00820, 0.00024–0.00132, and 0.00013–0.00051 μg/kg bw per day, respectively. It was observed that the exposure values reported in literature were lower than the values obtained in our study. It is thought that this difference could be due to higher daily tomato paste consumption than daily pepper consumption.

Aflatoxins are resistant to food processing methods because of their high chemical and thermal stability. This makes it extremely difficult to achieve zero exposure to aflatoxins by consuming contaminated food. Risk assessments play a vital role in managing and reducing potential risks associated with consumption of aflatoxins, thereby ensuring food and consumer safety (Bhardwaj *et al.,* 2023).

An EFSA's (2020) report stated that the average dietary exposure to AFB1 for adults was estimated as 0.22–0.49 ng/kg bw per day (LB) and 1.35–3.25 ng/kg bw per day (UB), while the 95th percentile of dietary exposure to AFB1 for adults, this range was approximately 0.62–1.36 ng/kg bw per day (LB) and 2.76–6.78 ng/kg bw per day (UB). A scientific committee on food of EFSA (2007) has warned that even exposure to aflatoxins at a level as low as 1 ng/kg bw per day may increase the risk of developing liver cancer.

Akhtar *et al.* (2020) reported that the highest exposure (3.29 ng/kg bw/day) was observed incase of female consumers aged > 24 years through the consumption of unbranded spices, while the lowest exposure (0.31 ng/kg bw/day) was observed by the intake of branded spices in case of males aged 9–14 years.

Health risk assessment

Table 4 shows long-term exposure to AFB1 via consumption of branded/unbranded tomato paste and pepper paste samples in Türkiye.

Table 3. Long-term exposure to AFB1 via consumption of tomato paste and pepper paste samples in Türkiye.

		Mean of AFB1 (μg/kg)				EDI (mean intake of AFB1) (ng/kg bw/day)			
Paste type	Paste consumption (g/day)*	LB	MB	UB	95th Percentile	LB	MB	UB	95th Percentile
Tomato paste	17.40	0.27	0.68	1.10	1.84	0.0632	0.1610	0.2588	0.4344
Pepper paste	1.96	0.51	0.86	1.21	2.84	0.0135	0.0228	0.0321	0.0756

*Daily paste consumption in Türkiye.
AFB1: aflatoxin B1; EDI: estimated daily intake; LB: lower bound; MB: middle bound; UB: upper bound; 95P: 95th percentile.

Table 4. Long-term exposure to AFB1 via consumption of tomato paste and pepper paste samples in Türkiye.

Paste type	MOE				HCC			
	LB	MB	UB	95th percentile	LB	MB	UB	95th percentile
Tomato paste	6332	2484	1545	920	0.00139	0.00354	0.00569	0.00956
Pepper paste	29,576	17,519	12,445	5290	0.00030	0.00050	0.00071	0.00166

MOE: margin of exposure; HCC: hepatocellular carcinoma; LB: minimum value of positive samples. MB: mean of positive samples; UB: maximum value of positive samples.

The EFSA (2020) Panel on Contaminants in the Food Chains demonstrated that MOE < 10,000 would be of concern for public health and might be considered a priority for risk management actions. The estimated MOE values (LB and UB) for AFB1 in the Turkish population through consumption of tomato and pepper pastes were estimated as 6332–1545 and 29,576–12,445, respectively. While the average MOE values calculated for the Turkish population were below the safe threshold of 10,000 for tomato pastes and above 10,000 for pepper pastes.

Based on the mean potency estimates and a prevalence of 0.2% of liver cancer, the EFSA Panel on Contaminants in Food Chains (CONTAM Panel) determined that the risk of cancer from average dietary exposure to AFB1 in adults ranged from 0.004 to 0.057 aflatoxin-induced cancers per 100,000 people/year. Based on UB potential estimates and a prevalence of 7.6% of liver cancer, the same panel estimated the worst-case potential risk of cancer from dietary exposure to AFB1 in adults ranged from 0.019 to 0.286 aflatoxin-induced cancers per 100,000 people/year (EFSA, 2020).

The estimated average HCC values for AFB1 (LB-UB) for tomato paste and pepper paste consumption inthe Turkish population ranged between 0.00139 and 0.00569, and 0.00030 and 0.00071 cases per 100,000 people/year, respectively. These values werebelow the estimated HCC value per 100,000 people/year for the average AFB1 related to the consumption of tomato and pepper pastes by the Turkish population. Considering the risk factors of aflatoxin exposure by consuming tomato paste and pepper paste alone, it may not pose a significant problem. However, it is important to remember that Turkish consumers are exposed to aflatoxin contamination from various other food sources, particularly grains (Oktay Basegmez, 2019; Özlü, 2024).

Özlü (2024) determined that the estimated MOE values (LB-UB) for AFB1 in the Turkish population (excluding children aged 0–14 years) for consumption of red pepper flakes were estimated as 37.537–2.103. According to Öztekin and Karbancıoğlu-Güler (2022), red pepper had the MOE values of 977 for AFB1 and 909 for AFT. The intake of AFB1 and AFT at UB could lead to 0.0058 and 0.0062 liver cancer cases per 100,000 people per year, respectively. Taghizadeh *et al.* (2023) investigated the risk of oral exposure to mycotoxins from spices among Iranian consumers and concluded that the oral consumption of the analyzed samples did not pose a carcinogenic risk concerning aflatoxin exposure. However, a study conducted in Pakistan by Akhtar *et al.* (2020) discovered that the MOE values for aflatoxins through spice consumption indicated that all age groups consumed aflatoxins above the threshold level.

Conclusions

The results of the present study concluded the following: (1) The average AW levels of unlabeled tomato and pepper pastes were statistically lower than those of commercially produced tomato paste and pepper paste samples ($p \leq 0.001$). (2) Although 15 (9.4%) of the 160 tomato paste samples analyzed were contaminated with AFB1, no sample exceeded the legal limit of 5 μg/kg set by the EU and Türkiye government. (3) MOE- and AFB1-related cancer cases showed that consumers are posed to potential risk of cancer. (4) Evaluation of data from this study demonstrated that although the AFB1 levels were relatively low, the EDI, MOE, and HCC values were high. It was assumed that that this situation could be due to the high daily consumption of tomato paste. (5) The legal limits for AFB1 contamination in foods must be reduced to the lowest possible levels. (6) Preventing AFB1 contamination in foods having high daily consumption is crucial for public health. Finally, (7) Growth of aflatoxin, a significant concern for the food industry, can be controlled by implementing effective, sustainable, and globally applicable pre-harvest prevention strategies through favorable agricultural and production practices at all stages of cultivation, refinement, transport, and storage.

Author Contributions

M.A.A. and H.Ö. prepared methodology and conceptualization, conducted the experimental work. M.A.A. wrote the original draft. M.A. reviewed and edited the original article.

Conflict of Interest

The authors declare no conflict of interest.

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