Occurrence, dietary exposure and risk assessment to aflatoxins in red pepper flakes from Southeast of Türkiye

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Abstract

Aflatoxins contamination of foods is a global issue that severely threatens human health. This study aimed to measure total aflatoxin (AF) (aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2)) levels in red pepper flakes samples and to evaluate the potential dietary exposure and cancer risk to aflatoxin in the southeast of Türkiye. A total of 90 red pepper flake samples were collected from local markets Gaziantep, Şanlıurfa, and Hatay provinces, Türkiye. Total AF in red pepper flakes was determined by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). Then, the margin of exposure (MOE) and cancer risk were calculated. AFB1 and total AF were detected in red pepper flake samples at 98.89% and 100%, respectively. The number of red pepper flake samples exceeded the legislation limits for AFB1 was four, and there was only one sample for total AF. It was determined that AFB1 exposure could pose a risk to public health for Turkish adults (MOE < 10,000). Although AF exposure seems to be higher due to the high consumption of red pepper flakes in some regions of Türkiye, the risk of exposure and cancer may decrease because of complying with the recommendations of the dietary guidelines. The findings provide new insights into exposure to total AF by consuming red pepper flakes.

Keywords: aflatoxins; dietary exposure; dietary risk assessment; HPLC-FLD; red pepper flakes

Introduction

Dried red pepper is among the most popular and widely consumed spices worldwide. The three countries with the highest red pepper production in the world in 2021 were China, Türkiye, and Indonesia, respectively. In Türkiye, dried red pepper produced approximately 15,800 tons in 2021, and 5,168 tons were exported (FAOSTAT, 2021). Dried red pepper (commonly known as paprika and chili in English) is obtained by drying the fresh ripe product from the red pepper plant of the *Capsicum spp.* (the most common type being *Capsicum annuum* L.), a member of the *Solanaceae* family (Ok *et al.,* 2015; Singh and Cotty, 2017). Dried red pepper has been a widespread ingredient in foods for centuries because of its appealing color, distinctive flavor, sharpness, and stimulating taste (Lee *et al.,* 2020).

Inadequate hygiene measures during the stages of red pepper production involving harvesting, drying, transportation, and storage can result in mycotoxin formation (Aydin *et al.,* 2007). Mycotoxins are natural toxins that several mold species produce (Khazaeli *et al.,* 2017). The most dangerous and well-researched mycotoxins are aflatoxins (Sowley, 2016). Aflatoxins, primarily *A. flavus* and *A. parasiticus*, are highly toxic secondary metabolites produced by various species of *Aspergillus* section Flavi via the polyketide pathway (Xiong *et al.,* 2022; Udomkun *et al.,* 2017).

The International Agency for Research on Cancer (IARC) has categorized aflatoxin exposure as Group 1, which is human carcinogenic (hepatocellular cancer). The type AFB1, in particular, is the most common and harmful food toxin (IARC, 2012). Although aflatoxins have been primarily associated with cancer, it has become increasingly apparent that aflatoxins also cause a variety of other acute and chronic diseases, many of which are severe (Benkerroum, 2020).

Aflatoxin contamination of food is a more prominent issue, particularly in countries with tropical and subtropical climates where humidity and temperature are high (Al-Zoreky and Saleh, 2019; Ayofemi Olalekan Adeyeye, 2020). Most often, aflatoxin contamination continues after crop harvest and throughout the post-harvest storage period (depending on factors such as humidity, temperature, drying, and ventilation) (Abrar *et al*., 2023). Even though Türkiye is relatively favorable in terms of climate zone, due to mold growth and toxin formation, there may be losses in the field, warehouses, or processed products from time to time.

Many countries and international organizations set the maximum permissible amounts of aflatoxins in various food items. This level may vary depending on the type of product and whether the country exports or imports the impacted food items. The national legislation on mycotoxins in Türkiye is in accordance with the regulations of the European Union (European Commission, 2010). According to Turkish law (Turkish Codex), the highest AFB1 level for red peppers (*Capsicum* spp., their dried fruits, sweet or spicy red peppers in whole or powdered form) can be 5 ppb, while the levels for total AF are 10 ppb (TFC 2011).

Based on the reports published by the Rapid Alert System for Food and Feed (RASFF) from January 2020 to July 2023, it is revealed that 589 of those contain mycotoxins, while 453 (92.2%) of them are related to aflatoxins. 33 of the 44 notifications concerning mycotoxins in the category of "herbs and spices" were related to aflatoxins. There were no red pepper samples among the five, which were of Türkiye origin (RASFF, 2023)

Due to the aflatoxins being genotoxic carcinogens, their levels within food items should be monitored and reduced by adhering to the as low as reasonably achievable (ALARA) principle to keep aflatoxin exposure at a minimum (EFSA, 2007). In developing countries where food regulations are not implemented or improperly applied, dietary exposure to aflatoxins could lead to specific health hazards (Ismail *et al.,* 2021). Chronic dietary exposure to certain mycotoxins found in spices has been established in some nations such as Spain (Cano-Sancho *et al.,* 2013), Ethiopia (Hunduma Tolera *et al.,* 2020), Serbia (Udovicki*et al*, 2021), Lebanon (Al Ayoubi *et al.,* 2021), and Pakistan (Naz *et al.,* 2022).

Although there are many studies on total AF contamination of red pepper flakes in Türkiye (Golge *et al.,* 2013; Ozkan *et al.,* 2015; Atasoy *et al.,* 2017; Acaroz, 2019), there have been very few studies regarding how spice consumption impacts Turkish society's exposure to aflatoxin (Kilic *et al.,* 2018; Kabak, 2021; Oztekin and Karbancioglu-Guler, 2022). This study highlights the daily consumption of red pepper in the human diet and the potential of AFB1 contamination, which is attributable to the risk of developing liver cancer. This study aimed to measure total AF (AFB1, AFB2, AFG1, AFG2) levels in red pepper flake samples and to evaluate the potential dietary exposure and cancer risk to aflatoxin in the southeast of Türkiye.

Materials and Methods

Sample collection

The provinces of Gaziantep, Şanlıurfa, and Hatay, where the study was carried out, are in the southeastern of Türkiye and are the major trade and industrial centers of southeastern Anatolia. The southeastern of Anatolia, which has a continental climate, has hot and dried summers and rarely cold winters. Most of the red pepper flake production in Türkiye is made in this region and is consumed a lot in this region.

A total of 90 red pepper flakes samples were collected from local markets that sell wholesale to different regions of Türkiye. For this reason, the number of samples was kept limited. Between April and October 2021, of the 90 samples of red pepper flakes, 30 came from Gaziantep, 30 came from Şanlıurfa, 30 came from Hatay. The samples were stored in resealable plastic pouches at +4°C until analyzed.

Chemicals and reagents

The aflatoxin standard solution was purchased from Supelco (Bellefonte, PA; Aflatoxin B and G mix, Cat. No. 46304-U). In 1 mL of methanol, each standard mix contains 1 µg of AFG1, AFB1, and 0.3 µg of AFG2, AFB2. Solvents (HPLC grade) and reagents (of 99% purity), including methanol, acetonitrile, potassium bromide, nitric acid, and phosphate-buffered saline (PBS), were purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q Plus system (Millipore, Brussels, Belgium). The IACs AflaPrep was purchased from R-Biopharm Rhone (Glasgow, Scotland).

Analytical quality parameters

To determine the method's validity; linearity, recovery, limit of detection (LOD), and limit of quantification

(LOQ) were evaluated. In order to evaluate linearity, solutions were prepared from the stock solution with concentrations of 1.5, 2.5, 5, 10, and 20 ng/mL for AFB1 and AFG1, and 0.25, 0.625, 1.25, 2.5, and 5 ng/mL for AFB2 and AFG2, respectively. By calculating the electronic area of the peak obtained for each concentration, 5-point calibration curves were drawn. Selectivity was examined by adding toxins to positive samples and then observing the rise in toxin peaks. The retention times of the peaks in samples were examined as well to ensure the results corresponded to one another. Recovery was assessed in three copies, the observed values were reported as a percentage compared to the spike levels in the uncontaminated sample. The LOD and LOQ limits of the chromatographic procedure for spiked samples were determined at signal-to-noise ratios of 3:1 and 10:1, respectively. The linear regression results used to calculate the aflatoxin content of the samples were expressed as µg/kg (ppb).

Extraction and purification

Aflatoxin extraction from the red pepper samples was based on the AOAC Official Method of Analysis 999.07 with minor modifications (Stroka *et al.,* 2019).

25 g of the samples were weighed and mixed with 2 g of NaCl and 100 mL of 80% methanol in an Erlenmeyer for 5 minutes using a high-speed mixer until homogenization. After extraction, the resulting mixture was run through Whatman No. 1 filter paper. 4 mL of the filtrate was combined with 16 mL of the phosphate buffer solution. The extract was then passed through an immunoaffinity column containing Aflaprep AFB1, AFB2, AFG1, and AFG2 antigens at a 2 mL/min flow rate. The immunoaffinity column was rinsed with 10 mL of PBS solution and 10 mL of water after the mixture had been run through it. The column was then quickly dried with air. After waiting for one minute, 1 mL of methanol was passed through the column for the second time, and positive pressure was created with the help of air so the methanol used in the separation was completely removed. The extract was then diluted with 1 mL of distilled water and kept at +4°C until HPLC-FLD analysis.

HPLC conditions and quantification

HPLC system (Shimadzu LC-20AD Prominence, Shimadzu Corp, Kyoto, Japonya) was used to determine the AFB1, AFB2, AFG1, and AFG2 levels in the red pepper, and an FLD (RF10AXL) detector was used for quantitative analysis. In order to detect AFB1 and AFG1 in the detector, a derivatization cell (Kobra Cell) was added between the HPLC and the detector, through bromination, the electrochemical derivatization was performed. Aflatoxin detection with an FLD detector was carried out for 30 minutes at 30°C within the 365–435 nm (excitation–emission) wavelength range. As the mobile phase for separation, a mixture of purified water, acetonitrile, and methanol (6:2:3/v:v:v) was used, and the flow rate was set to 1 mL/min. 120 µl potassium bromide and 350 µl of nitric acid (4 M) solution were added for every liter of the mobile phase mixture. The mobile phase was passed through a 0.45 µm PTFE filter before usage. The flow rate was set for 1 ml/min, the furnace temperature at 30°C and the upper limit of the column pressure at 620 bar. For each sample, 50 µl of extract was injected twice into the HPLC. In order to rebalance the colon, a 2-minute gap was allowed between injections.

Estimated daily intake

Mycotoxin formation data in foods and food consumption data are evaluated jointly for the estimation of dietary exposure, so mycotoxin formation data should be evaluated accordingly. The most popular methods in dietary evaluations are the use of lower bound (LB) and upper bound (UB) values. However, to achieve more accurate results, the European Food Safety Authority (EFSA) suggests handling left-skewed data using the substitution method, considering the percentage of left-censored data in all data (up to 60% of uncensored data). The standard approach is as follows when samples have a significant amount of left-censored data: (1) assign a value of zero for the LB estimate, (2) assign LOD/2 or LOQ/2 for the middle bound (MB) estimate, and (3) assign LOD or LOQ for the UB estimate (EFSA, 2010). Our study, however, used the minimum value for LB and the maximum value for UB since the data were skewed to the right rather than the left.

In accordance with the method suggested by FAO/WHO, the estimated daily food-borne aflatoxin intake values based on body weight were calculated from the formula below.

$$EDI = Di \times \frac{Mi}{W} \quad (1)$$

Di represents the daily consumption (g/day) of the mean product quantity consumed by the population per day,

Mi stands for the average AFB1 concentration (µg/kg), and W is for body weight (kg).

The estimated daily red pepper consumption per capita in Türkiye was determined to be 0.55 g based on the FAOSTAT data (FAOSTAT, 2021). When calculating dietary exposure of adults to AFB1 and total AF, the average bw for the adult Turkish population in 2021 (73.7 kg) was used for the exposure estimates according to the National Institute of Statistics (TUIK, 2023).

Health risk assessment

Due to the genotoxic and carcinogenic potential of aflatoxins, exposure levels should be as low as permissible. Therefore, no tolerable daily intake has been determined. MOE values and cancer potency estimates were utilized to quantify the health risk associated with aflatoxin levels in consumed red peppers.

MOE approach was used the BMDL10 (benchmark dose lower confidence limit 10) and EDI of AFB1. The MOE was calculated using a BMDL10 value of 0.4 µg/kg bw day used for AFB1, which the EFSA recommends. An MOE value of 10,000 or above is considered a low risk for public health (EFSA, 2020).The MOE value is calculated using the equation given below:

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

The cancer risk associated with aflatoxin exposure in the analyzed red pepper flake samples was calculated using Equations 3, 4, and 5 (Udovicki *et al.,* 2021). This calculation estimates the cancer risk per 100,000 population, which is a product of the EDI value and the mean hepatocellular carcinoma (HCC) potency figure derived from the individual potencies of the Hepatitis B surface antigen (HBsAg) (HBsAg-positive and HBsAg-negative groups). The $HBsAg^+$ value of 4% for Turkish adults was adopted, based on the rate reported in a recent study in Türkiye by Özkan (2018). The median strength and population risk were estimated according to the following formulae.

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

$$Pcancer = 0.01 \times 0.96 + 0.3 \times 0.04 = 0.022 \quad (4)$$

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

$$HCC = EDI \times \underline{Pcancer} \quad (5)$$

Results

Method performance

The extraction and quantitation stages were performed in duplicate for each sample. Standard solutions used for the calibration curve were injected into the sample injections at regular intervals to verify the retention times. The retention time for AFB1, AFB2, AFG1, and AFG2 was determined to be 12.012, 9.986, 9.027, and 7.636 min, respectively. No interference peaks were observed during the retention time as the analytes were processed and chromatograms showed fine resolution.

The calibration line was adjusted according to the standards and results were given according to the working range. All analytes showed correlation coefficients greater than 0.98, which is in good agreement with the performance criteria of Commission Regulation No 401/2006 (European Commission 2006) concerning quantitative mycotoxin analysis methods. The recovery, LODs, and LOQs of the method are presented in Table 1.

Occurrence of aflatoxins

Total aflatoxin contents of 90 red pepper samples from the Gaziantep, Şanlıurfa, and Hatay provinces of Türkiye were analyzed by HPLC in 2 repetitions for three parallel samples. Table 2 presents the aflatoxin formation of the red peppers. AFB1 was determined in all except 1 of the 90 samples analyzed (obtained from Hatay province). AFB2 was detected in 28 of 30 samples from Hatay, 27 from Gaziantep, and 16 from Şanlıurfa. In Gaziantep, AFG1 and AFG2 were also detected in 1 and 2 samples, respectively. Among those samples contaminated with AFB1, none of them were identified to be above the maximum permissible limit (>5 µg/kg) by the European Union and Turkish Food Codex, except for three samples in Hatay province and 1 sample in Gaziantep province. Only one sample had a total AF concentration (26.63 µg/kg) above the maximum permissible limit of 10 µg/kg. According to these results, 98.89% of the samples (89 samples) are suitable for consumption due to their total AF concentration.

Estimated daily intakes of aflatoxin by adults in Türkiye

The min, max, mean and 95th percentile concentrations of AFB1 and total AF in red pepper flakes and chronic exposure estimates calculated from daily consumption patterns of red pepper flakes per capita in Türkiye are summarised in Table 3.

Table 1. Validation of aflatoxins determination by HPLC analysis.

Analyte	Range (µg/L)	Linearity (r^2)	Recovery (%)	LOD	LOQ
AFB1	1.5–20	0.9997	100.45	0.1	0.5
AFB2	0.25–5	0.9998	98.96	0.03	0.1
AFG1	1.5–20	0.9999	98.37	0.06	0.02
AFG2	0.25–5	1	98.38	0.02	0.09

Table 2. AFB1, AFB2, AFG1, and AFG2 contamination in red pepper flakes.

Aflatoxin	Level	Province			
		Şanlıurfa (N=30)	Gaziantep (N=30)	Hatay (N=30)	Total (N=90)
AFB1	Positive[a] N (%)	30 (100)	30 (100)	29 (96.7)	89 (98.89)
	>5 µg/kg[b] N (%)	ND	1 (3.33)	3 (10.0)	4 (4.44)
	Mean±SD(µg/kg)[c]	1.67±0.59	3.04±4.39	2.38±1.69	2.36±2.77
	Range µg/kg[d]	1.43-4.42	1.44-25.54	1.43-8.06	1.43-25.54
AFB2	Positive[a] N (%)	16 (53.33)	27 (90.00)	28 (93.33)	71 (78.89)
	>5 µg/kg[b] N (%)	ND	ND	ND	ND
	Mean±SD(µg/kg)[c]	0.03±0.03	0.22±0.47	0.10±0.11	0.13±0.30
	Range µg/kg[d]	0,0003-0,073	0,001-2,30	0.001-0.37	0.0003-2.30
AFG1	Positive[a] N (%)	ND	1 (3.33)	ND	1 (1.11)
	>5 µg/kg[b] N (%)	ND	ND	ND	ND
	Mean±SD (µg/kg)[c]	-	0.21±0.00	-	0.21±0.00
	Range µg/kg[d]	-	0-0.21	-	0-0.21
AFG2	Positive[a] N (%)	ND	2 (6.66)	ND	2 (6.66)
	>5 µg/kg[b] N (%)	ND	ND	ND	ND
	Mean±SD (µg/kg)[c]	-	0.03±0.03	-	0.03±0.03
	Range µg/kg[d]	-	0.008-0.056	-	0.008-0.056
Total AF	Positive[a] N (%)	30 (100)	30 (100)	30 (100)	90 (100)
	>10 µg/kg[b] N (%)	ND	1 (3.33)	ND	1 (1.11)
	Mean±SD (µg/kg)[c]	1.69±0.61	3.24±4.61	2.40±1.76	2.44±2.91
	Range µg/kg[d]	1.43-4.48	1.45-26.63	0.27-8.32	0.27-26.63

[a]At detectable limit, >LOD; [b]Exceed European and Turkish legal limit; [c]Positive samples; [d]Min-Max; N: number of samples; ND: Not detected.

Table 3. EDI (ng/kg bw per day) for AFB1 and total AF for the Turkish adult population across foods containing red pepper as an ingredient (except 0–14 aged children group).

	Concentrations (µg/kg)				EDI*			
	LB	MB	UB	P95	LB	MB	UB	P95
AFB1	1.43	2.36	25.54	5.35	0.0107	0.0176	0.1902	0.0398
Total AF	0.27	2.44	26.63	5.97	0.0020	0.0182	0.1984	0.0445

LB (lower bound): minimum value of positive samples; MB (middle bound): mean of positive samples; UB (upper bound): maximum value of positive samples; P95: 95th percentile.
*:Daily consumption amount of red pepper flakes in Türkiye 0.55 g (FAOSTAT 2021).

Health risk assessment

MOE and HCC values for AFB1 of foods containing red pepper flakes are presented in Table 4. The estimated MOE values (LB-UB) for AFB1 of the Turkish population (excluding children 0–14 years) for red pepper flake consumption were 37.537-2.103 and 202.836-2.016, respectively. While the average MOE values calculated for the Turkish adult population were above 10,000, the maximum values were below 10,000. An MOE value of less than 10,000 indicates that consuming aflatoxin-contaminated foods is a public health risk (EFSA 2020). The estimated average HCC values for AFB1 for red pepper flake consumption for the Turkish population ranged between 0.00023-0.00418 cases per 100,000 people/year, respectively.

Discussion

Occurrence of aflatoxins

The formation of aflatoxins is one of the most prominent concerns encountered in red pepper flakes and its dried end-products. In the present study, AFB1 concentration in 4 of the red pepper flakes samples available in three

Table 4. MOE and HCC risk values were calculated from mean dietary exposure to AFB1 for the Türkiye population across foods containing red pepper (except the 0-14 aged children group).

MOE				HCC*			
LB	MB	UB	P95	LB	MB	UB	P95
37,537	22,736	2,103	10,041	0.00023	0.00039	0.00418	0.00088

*HCC: hepatocellular carcinoma cases/10^5 person/year.
LB (lower bound): minimum value of positive samples; MB (middle bound): mean of positive samples; UB (upper bound): maximum value of positive samples; P95: 95th percentile.

provinces of south-eastern Türkiye was determined to be below the maximum permissible limits set by the European Union and Turkish Food Codex. In contrast, only one sample exceeded these limits. Red pepper is one of the most popular and widely consumed spices in Türkiye, particularly in the southeastern region. This study revealed that all samples were contaminated despite the low levels of aflatoxin contamination in red pepper flakes. Therefore, these toxins constantly threaten the spice industry and consumers in the subtropical regions of Türkiye.

In the present study, although all 90 red pepper flakes samples showed total AF contamination in the range of 0.27-26.63 µg/kg, only one (1.11%) was above the legal limits. When similar researches in Türkiye are examined, Colak *et al.,* (2006) reported that 12 out of 60 red pepper samples were contaminated with total AF between 0.7-46.8 µg/kg (20%), Karaaslan and Arslanğray (2015) reported that 13 out of 42 red pepper flakes samples were contaminated with total AF (31.0%), Demircioğlu and Filazi (2010) reported that 11 out of 49 red pepper samples were contaminated with total AF (22%) while Golge *et al.,* (2013) recorded that 35 out of 182 red pepper samples (19.2%) had total AF contamination above the legal limits.

On the other hand, according to the results of this study, although 89 (98.9%) red pepper samples were contaminated with AFB1 within the range of 1.43-25.54 µg/kg, only four (4.44%) of them exceeded the legal limits. In similar studies conducted in Türkiye, Aydin *et al.,* (2007) reported that 18 (18%) of 100, Ardic *et al.,* (2008) reported that 11 (14.7%) of 75, and Golge *et al.,* (2013) reported that 50 (27.5%) of 182 red pepper samples had AFB1 contamination levels above the legal limit.

According to various studies conducted on red peppers across the globe, aflatoxin levels were observed to be considerably high. Abdulkadar *et al.,* (2004) reported that 66.6% of samples from Qatar were contaminated with aflatoxins ranging between 5.60 and 69.28 µg/kg. Romagnoli *et al.,* (2007) determined the incidence (45.7%) of total AF in chili pepper samples in Italy in the range of 0.57-30.7 µg/kg. Another research conducted in Qatar revealed that the maximum contamination levels of AFB1 and total AF in chili powder samples were recorded as 69.28 µg/kg and 71.01 µg/kg, respectively (Hammami *et al.,* 2014). Barani et al., (2016)conducted a study in Iran and reported that AFB1 contamination in 32 (88.9%) and total AF contamination in 25 (32.9%) of 36 red pepper samples were higher than the maximum permissible levels in Iran and Europe. Contrary to these studies, Kim *et al.,* (2020) in South Korea reported that only 15 (5.77%) of 260 red pepper samples were contaminated within the 0.20-2.33 µg/kg range.

Estimated daily intakes of aflatoxin by adults in Türkiye

In the present study, the average EDI values for AFB1 and total AF of red pepper-containing food products for Turkish individuals were reported to be 0.0176 and 0.0182 ng/kg bw per day, respectively, and regarding food items containing red pepper, Kabak (2021) determined the average EDI values for AFB1 and total AF as 0.044 and 0.047 ng/kg bw per day, respectively, while Oztekin and Karbancioglu-Guler (2022) observed them as 0.174 and 0.187 ng/kg bw per day, respectively. The values determined in these two studies conducted in Türkiye were observed to be relatively higher than the values obtained from the present study.

Aflatoxins are particularly difficult to eliminate, even after the processing of food, due to their high chemical and thermal stability. This makes it almost impossible to achieve zero exposure by consuming contaminated food. Risk assessments are vital in managing and mitigating potential hazards associated with aflatoxin consumption to ensure food and consumer safety (Bhardwaj *et al.,* 2023).

In Ethiopia, three-fourths (75%) of the total aflatoxins detected in red pepper were greater than the maximum permissible levels set by the European Union (10 µg/kg). The MOE values for all total AF were far from the safe margin (<10,000), indicating potential health risks due to red pepper consumption (Adunga *et al.,* 2022).

EFSA's 2020 report indicated that the average dietary exposure to AFB1 for adults was estimated to be between

0.22-0.49 (LB) and 1.35-3.25 (UB) ng/kg bw day, while for the 95th percentile of dietary exposure to AFB1, this range was approximately 0.62-1.36 (LB) and 2.76-6.78 (UB) ng/kg bw day (EFSA, 2020). According to the Scientific Committee on Food, exposure to aflatoxins at levels as low as 1 ng/kg bw day could increase the risk of developing liver cancer (EFSA 2007).

In the study conducted by Kabak (2021), it was reported that the average chronic dietary exposure to AFB1 and total AF in the Turkish adult population ranged between 0.402-0.465 ng/kg bw per day (LB-UB) and 0.448-0.574 ng/kg bw per day (LB-UB), respectively. In addition, the 95th percentile dietary exposure to AFB1 and total AF for Turkish adults was determined to be 1.19 and 1.29 ng/kg bw per day, respectively. However, the mean and 95th percentile contributions of chili pepper regarding dietary exposure to AFB1 were 10.8% and 17.8%, respectively, while for total AF exposure, the figures were 10.5% and 16.4%.

Adults primarily consume red pepper. No consumption data on red pepper were collected for children or adolescents in Türkiye. Therefore, consumption estimates of red pepper added to foodstuffs were calculated for the total population of Türkiye, excluding the 0-14 age group. The estimated daily red pepper consumption per capita in Türkiye was determined to be 0.55 g based on the FAOSTAT data (FAOSTAT, 2021). It is crucial to conduct detailed consumption research to obtain accurate dietary aflatoxin exposure estimates for the population through the development of food consumption databases.

It was determined in the study conducted by Kiliç *et al.,* (2018) that the consumption of 20 g of red pepper by an adult in Türkiye (72.8 kg bw) leads to an AFB1 daily intake of 1.5 ng/kg bw (average AFB1 result of the 33 samples), which is about 150% higher than the tolerable daily intake (1 ng AFB1/kg bw).

Even though red pepper has a higher frequency and concentration of contamination than other food products, its lower consumption rate contributes less to dietary exposure to AFB1 and total AF. Nonetheless, Bhardwaj *et al*., (2023) reported in a study that the herbs and spices category (especially chili and nutmeg) yielded the third-highest number of aflatoxin occurrences. Therefore, dietary intake of aflatoxins through the consumption of red pepper poses a substantial food safety concern for consumers and a potentially significant health risk, including HCC.

Health risk assessment

Based on the mean potency estimates and a prevalence of 0.2%, the CONTAM Panel determined that the cancer risk from average dietary exposure to AFB1 in adults ranged from 0.004 to 0.057 aflatoxin-induced cancer per 100,000 people/year. Based on UB potential estimates and a prevalence of 7.6%, the same panel estimated the worst-case potential risk of cancer from dietary exposure to AFB1 in adults to range from 0.019 to 0.286 aflatoxin-induced cancers per 100,000 people/year (EFSA, 2020). These values are below the estimated HCC value per 100,000 people/year for the average AFB1 related to the adult Turkish population's consumption of red pepper flakes.

Although there have been numerous studies on the mycotoxin levels in red pepper, only recently have studies on dietary exposure and risk assessment been conducted, resulting in the matter being evaluated from various perspectives. Dietary intake of Group 1 carcinogen aflatoxins, known to cause hepatocellular carcinoma, is the primary route of total AF exposure in humans (Chen *et al.,* 2022). According to Oztekin and Karbancioglu-Guler (2022), red pepper had MOE values of 977 and 909 for AFB1 and total AF, respectively, and the intake of AFB1 and total AF at LB-UB could result in 0.0057-0058 and 0.0060-0.0062 liver cancer cases per 100,000 people/year, respectively.

The etiology of aflatoxin contamination of chili is complex and may vary with region. Therefore, the exposure and cancer risk of people living in different regions may vary (Singh and Cotty, 2019). While it was determined that the consumption of spices was not a carcinogenic risk in terms of aflatoxin exposure in the study conducted by Taghizadeh *et al.* (2023) in Iran, it was determined that the consumption of spices was posing a risk of exposure in a study conducted Akhtar *et al.* (2020) in Pakistan.

Conclusion

The present study found that the incidence of total AF contamination in red pepper flakes sold in southeast Türkiye was not very high. Based on this subject in the related literature, Türkiye encountered a high rate of total AF notifications in recent years. As a result, it can be concluded that effective control measures implemented by the official authorities have contributed to a decrease in contamination levels. Nonetheless, controlling these toxins' health threats and routinely inspecting marketed spices for regulatory compliance is necessary. With the implementation of effective, sustainable, and globally applicable pre-harvest prevention strategies through favorable agricultural and production practices in all stages of cultivation, refinement, transport, and storage, the growth of *A. parasitics* and the total AF contamination, a significant concern in the industry, can be taken under control.

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