

## ORIGINAL ARTICLE

# Determination of mold contamination and aflatoxin levels of the meat products/ingredients collected from Turkey market

Soner Cavus<sup>1</sup> | Fatih Tornuk<sup>2</sup> | Kemal Sarioglu<sup>1</sup> | Hasan Yetim<sup>3</sup> 

<sup>1</sup>Food Engineering Department, Erciyes University, Kayseri, Turkey

<sup>2</sup>Food Engineering Department, Yildiz Technical University, Istanbul, Turkey

<sup>3</sup>Gastronomy and Culinary Arts Department, Gelisim University, Istanbul, Turkey

## Correspondence

Hasan Yetim, Faculty of Fine Arts, Gastronomy and Culinary Arts Department, Gelisim University, 34315 Avcilar, Istanbul, Turkey.  
Email: hyetim@gelisim.edu.tr

## Abstract

This study aimed to highlight general safety conditions of meat products ( $n = 24$ , fresh meat, Turkish sucuk, sausage, and pastirma) and ingredients ( $n = 20$ , red paprika, black pepper, coriander, spice mix and fenugreek powder) sold in Turkey regarding mold and aflatoxin contamination. Total 55 mold species belonging to *Aspergillus* and *Penicillium* sp. were isolated while *A. flavus* and *A. niger* were the most prevalent ones. Aflatoxin B1 (AFB1) was detected in 50% ( $n = 12$ ) and 65% ( $n = 13$ ) of the meat products and ingredients while 25% and 43% of sucuk and pastirma samples had mold counts exceeding the limits of Turkish Food Codex, respectively. However, one red paprika sample had AFB1 and total aflatoxin levels over the limitations of Turkish Food Codex and European Commission. It was concluded that Turkish meat products/ingredients were generally safe for consumption.

**Practical applications:** There is an increasing attention on mycotoxin contamination of foodstuffs in terms of consumer health as well as in international trade. Although especially spices are under the risk of mold growth and formation of mycotoxins, animal products such as beef and milk are also frequently exposed to mycotoxin contamination. In the meanwhile, national and international regulatory agents have established limits for the presence of aflatoxins in foodstuffs. Therefore, this study provides beneficial information about general safety conditions of several meat products and ingredients sold in Turkey market.

## 1 | INTRODUCTION

The presence of harmful compounds such as organic residues and microbial contaminants in foodstuff are among the most common health and food safety concerns in the world. Mycotoxins, toxic secondary metabolites produced by fungi, take an important part in food contaminants because it is estimated that approximately 25% of food crops are contaminated with significant levels of mycotoxins (WHO, 1999). Especially, crops grown in tropical and subtropical climatic conditions are more susceptible to mycotoxin production by fungi (Thompson & Henke, 2000).

All of the fungi species do not produce mycotoxins while the species belonging to *Aspergillus*, *Penicillium*, and *Fusarium* are known to synthesize them (Wagacha & Muthomi, 2008). Aflatoxin is the most common mycotoxin produced by *Aspergillus* species especially *A. flavus* and *A. niger* (Moss, 1998; Saleemullah, Iqbal, Khalil, & Shah, 2006). A number of health hazards including carcinogen, mutagen,

teratogen and hepatotoxic of aflatoxins have been reported (Ghasemi-Kebria et al., 2013; Speijers & Speijers, 2004). The kinds of aflatoxins, namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) are the metabolic products of the fungi formed by their activity in contaminated foods and feeds. Among these, AFB1 is known as the most carcinogenic and toxic one that is classified in the Group 1 carcinogens by International Agency of Research on Cancer (IARC) (Bennett & Klich, 2003; Paget et al., 2012). The presence of AFB1, AFB2, AFG1, and AFG2 in animal origin foods such as red meat, egg, and milk indicates the presence of aflatoxin-contaminated feeds of animal rations (Peraica, Domijan, Jurjević, & Cvjetković, 2002). Also, it is expected that the presence of mycotoxins most likely is the indication of low quality or contaminated spice and seasoning used in the meat products. Aflatoxins M1 and M2 (AFM1) and AFM2) are the metabolic products derived from AFB1 and AFB2 by activity of the body of lactating animals, respectively (Herzallah, 2009).

Several mycotoxins such as aflatoxin have been considered as inevitable contaminants of foods and feeds by Food and Drug Administration (Wagacha & Muthomi, 2008). Therefore, national and international agencies have been established limits for levels of contamination of aflatoxins in foods. According to Turkish Food Codex and European Commission Regulation (Regulation No. 1881/2006), maximum limits of AFB1 and total aflatoxins (AFB1 + AFB2 + AFG1 + AFG2) were approved as 5 and 10 ppb for spices/spice mixes. However, in the Turkish Food Codex, there is no legal regulation regarding mycotoxin limits in meat and meat products. In this study, it was aimed to determine mold and the aflatoxin presence and their levels, tested total 46 fresh meat, meat product and/or ingredients collected from Kayseri (Turkey) market.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

In this study, total 46 samples; fresh meat ( $n = 2$ ), meat products (sucuk = 11, sausage = 4, and pastirma = 7) or ingredients (spice mix = 4, red pepper = 5, black pepper = 5, coriander = 4, and fenugreek powder = 4) were collected from Kayseri market for microbiological assessment and aflatoxin determination. The meat samples were collected and transferred to laboratory in polyethylene bags in aseptic conditions while other samples (sucuk, sausage, pastirma, spice mix, black pepper, red pepper, coriander, and fenugreek powder) were purchased with their retail packaging materials. Microbiological analyses were performed at the same day of sample collection while in the case of aflatoxin determination, the samples were kept at  $-18^{\circ}\text{C}$  until the analyses.

Methanol, acetonitrile, nitric acid, ethanol, potassium bromide, dipotassium hydrogen phosphate, Tween 20, trisodium phosphate didecahydrate, potassium iodine, and sodium chloride were purchased from Merck (Germany). Aflatoxin Mix Kit-M (analytical standard in methanol) was provided from Supelco (Supelco, Bellefonte, PA).

### 2.2 | Microbiological analysis and identification of molds

Total mold counts of the meat products and the ingredients collected were analyzed using cultural microbiological counting methods. For this aim, 10 g of the sample was incorporated with peptone water and homogenized vigorously. Following preparation of the serial dilutions, 100  $\mu\text{L}$  of each appropriate dilution was spread plated onto Potato Dextrose Agar (PDA, Merck, Germany). The plates were incubated at  $25^{\circ}\text{C}$  for 4–5 days and the number of mold colonies were counted.

For the identification of mold colonies, different colonies grown on the petri plates were inoculated to PDA based on the 3-point inoculation method and the petri plates were incubated at  $25^{\circ}\text{C}$  for 5–10 days. From the petri plates inoculated with the highest dilutions, discrete colonies were selected. Then the colonies were subcultured on fresh petri plates. The mold species were identified based on their colonial (size, surface, appearance, texture, and color) and microscopic

characteristics (the presence or absence of cross-walls, and diameter of hyphae) (Harrigan & McCance, 1966).

### 2.3 | HPLC (High performance liquid chromatography) analysis of aflatoxins

AFB1, AFB2, AFG1, and AFG2 were analyzed using HPLC (HPLC, Agilent 1100, Waldbronn, Germany) equipped with fluorescent detector (FD, 8  $\mu\text{L}$  2 MPa, Germany). Aflatoxin analysis was composed of extraction and HPLC steps. Extraction of the aflatoxins from the samples was performed based on the method described by Senyuva and Gilbert (2005) with some modifications. For this aim, 12.5 g of sample and 1 g of NaCl were incorporated with 25 mL of distilled water and homogenized at 14,000 rpm for 1 min using Ultra Turrax (IKA, T18 Basic, Germany). Following the addition of 37.5 mL of methanol, the mixture was further homogenized for 2 min. Then the mixture was sequentially filtered with Whatman 2V and 934-AH filter papers. A 5 mL of the filtrate was incorporated with 15 mL of phosphate buffer solution (PBS) at pH 7.4 and the mixture was passed through the AflaTest immunoaffinity column (Vicom, Milford, CT) that was conditioned with 10 mL of PBS at a flow rate of 3 mL/min. The total aflatoxins were eluted with 0.5 and 1 mL of methanol (1 drop/s) followed by 0.5 and 1 mL of ultra-pure water (1 drop/s). Then the eluate (100  $\mu\text{L}$ ) was injected to HPLC.

In the case of HPLC analysis, mobile phase (water : methanol : acetonitrile, 60:20:20) was mixed with 120 mg/L potassium bromide and 100 ppm of 65% nitric acid. The mixture was pumped to the system with the flow rate of 1 mL/min. The HPLC column (C18, 5  $\mu\text{m}$ , 250 mm  $\times$  4.6 mm, Macherey – Nagel) was set to  $30^{\circ}\text{C}$  while the excitation and emission wavelengths were 333 and 460 nm, respectively. First, standard aflatoxin solutions were injected and the retention times were determined while the samples were analyzed. The analysis was finished in app. 25 min.

### 2.4 | HPLC method validation

Method validation of HPLC for aflatoxin determination was performed for fresh meat and sausage. First, standard solutions of the aflatoxin were prepared. A 1 mL of Aflatoxin Mix Kit-M (Supelco, Bellefonte, PA) standard containing AFB1, AFB2, AFG1, and AFG2 was incorporated with 9 mL of methanol. Then 40, 120, 200, 280, and 360  $\mu\text{L}$  of the mixtures were transferred into the vials containing 3,960, 3,880, 3,800, 3,720, and 3,640  $\mu\text{L}$  as well as 6 mL of ultra-pure water, respectively. The mixtures in the vials were finely homogenized and injected (100  $\mu\text{L}$ ) to HPLC to obtain the standardization curves of the aflatoxins. In order to determine the recovery rates (%), 1 mL of aflatoxin mix was incorporated with 9 mL of methanol. This mixture (50, 100, or 250  $\mu\text{L}$ ) was incubated for 1 hr at dark conditions after combining with 12.5 g of meat or meat ingredients which were free of aflatoxins. Then the standard HPLC procedures were performed. From the peaks of standard aflatoxins, limit of detection (LOD) and limit of quantification (LOQ) were calculated by the analysis of the matrix matched standards at the lowest calibration level, determined as the lowest concentration of the analytes that gave the chromatographic peaks at signal to noise (S/N) of 3 and 10, respectively.

**TABLE 1** Validation of HPLC method for determination of aflatoxin in meat products and ingredients

Aflatoxin type	LOD (ppb)	LOQ (ppb)	Recovery (%)	R <sup>2</sup>
AFB1	0.0063	0.021	60.643–63.508	0.9996
AFB2	0.0031	0.010	87.085–89.337	0.9994
AFG1	0.0087	0.029	66.411–92.533	0.9997
AFG2	0.0053	0.018	66.686–70.145	0.9992

Note. LOD, Limit of detection and LOQ, Limit of quantification.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Method performance

Performance of HPLC method for aflatoxin determination was assessed by several parameters including LOD, LOQ and % recovery, as seen in Table 1. All aflatoxin types were calibrated with good linearity indicated by R<sup>2</sup> levels higher than 0.999. Recovery rates of AFB1, AFB2, AFG1, and AFG2 of the blank samples ranged from 60.643% to 92.533%. The rates obtained by HPLC analysis of the aflatoxins were in conformity with the directions of European Commission (2006).

#### 3.2 | Mold counts and species

Table 2 shows mold counts of commercial fresh meat, sucuk, pastirma, and sausage samples. Meat and sausage samples were free of mold presence. However, 4 sucuk samples had mold populations ranging from  $1 \times 10^2$  to  $1 \times 10^3$  cfu/g while 57% of the pastirma samples

**TABLE 2** Mold counts of meat and meat products analyzed

Sample	Sample ID	Mold count (cfu/g)
Meat	1	<10
	2	<10
Sucuk	1	<10
	2	<10
	3	$1.5 \times 10^2$
	4	$1 \times 10^2$
	5	<10
	6	<10
	7	<10
	8	$1 \times 10^3$
	9	$1.5 \times 10^2$
	10	<10
	11	<10
Sausage	1	<10
	2	<10
	3	<10
	4	<10
Pastirma	1	<10
	2	$1.5 \times 10^2$
	3	$1.5 \times 10^2$
	4	$1 \times 10^2$
	5	$3 \times 10^2$
	6	<10
	7	<10

**TABLE 3** Mold counts of meat ingredients analyzed

Sample	Sample ID	Mold count (cfu/g)
Spice mix	1	$5.5 \times 10^2$
	2	$2 \times 10^2$
	3	$1.2 \times 10^2$
	4	<10
Red pepper	1	$6.3 \times 10^2$
	2	$1.7 \times 10^3$
	3	$1.4 \times 10^2$
	4	$2 \times 10^2$
Black pepper	1	$1.7 \times 10^3$
	2	$1 \times 10^2$
	3	$1 \times 10^2$
	4	$3.3 \times 10^2$
Coriander	1	$3.5 \times 10^2$
	2	$2 \times 10^2$
	3	$1.5 \times 10^2$
	4	$1 \times 10^2$
Fenugreek powder	1	$5.6 \times 10^2$
	2	$3 \times 10^2$
	3	$1 \times 10^2$
	4	<10

( $n = 4$ ) were mold positive. According to Turkish Food Codex Regulation on Microbiological Criteria, maximum limit for mold counts of meat products (heat processed or not) is  $1.0 \times 10^2$  cfu/g (Turkish Food Codex, 2009). As seen in Table 1, 27% and 43% of sucuk ( $n = 3$ ) and pastirma ( $n = 3$ ) samples had mold counts over the limits, respectively. In the case of microbiological assessment of the meat product ingredients analyzed, majority (90%) of the samples except for one spice mix (SM4) and one fenugreek powder samples (FF4) contained mold numbers ranging from  $1 \times 10^2$  to  $1.7 \times 10^3$  cfu/g (Table 3). However, all the samples were in conformity with the Turkish Food Codex regulations which approved the maximum yeast-mold limits as  $1.0 \times 10^4$  cfu/g.

As reported before, meat products have been commonly contaminated with molds. Núñez, Rodríguez, Bermúdez, Córdoba, and Asensio (1996) identified 32 species from the 519 mold isolates received from 42 dry-cured Iberian hams analyzed. They reported that more than 98% of the isolates were either *Penicillium*, *Aspergillus*, or *Eurotium* species. For most of the ripening time, the mold population was dominated by *Penicillium* sp. while *P. commune*, *P. chrysogenum*, and *P. expansum* were the species more frequently isolated. In another study conducted by Mizakova, Pipova, and Turek (2002), mold contamination of 70 fermented raw meat and flavoring samples as well as final products were analyzed at several monthly intervals. In their study, the presence of molds was observed in pork and beef used as a raw material in salami emulsions, and in five kinds of fermented raw meat products. In the results, *Penicillium* sp., *Acremonium* sp., *Mucor* sp., *Cladosporium* sp., and *Aspergillus* sp. were the most frequently isolated genera. Again, flavorings added to meat during the production of fermented raw meat products were heavily contaminated with molds. Asefa et al. (2009) collected 161 samples from the ripening and packaging stages of production with the aim of identifying molds

**TABLE 4** Mold species isolated from meat products and the ingredients

Sample	Sample ID	Mold species
Sucuk	3	<i>Scopulariopsis chartarum</i>
	4	<i>Cladosporium cladosporioides</i>
	8	<i>Penicillium jensenii</i>
	9	<i>Mucor circinelloides</i> f. <i>griseocyaneus</i>
Pastirma	2	<i>Scopulariopsis chartarum</i>
	3	<i>Cladosporium herbarum</i> , <i>Aspergillus niger</i>
	4	<i>P. jensenii</i>
	5	<i>Paecilomyces variotti</i>
Spice mix	1	<i>A. flavus</i>
	2	<i>A. niger</i>
	3	<i>A. flavus</i>
Red pepper	1	<i>A. flavus</i> , <i>A. niger</i>
	2	<i>A. flavus</i> , <i>A. niger</i>
	3	<i>A. flavus</i> , <i>A. niger</i> , <i>P. jensenii</i> , and <i>P. brevicompactum</i>
	4	<i>A. flavus</i> , <i>Scopulariopsis chartarum</i> , and <i>Acremonium</i> sp.
Black pepper	1	<i>A. flavus</i> , <i>A. ochraceus</i>
	2	<i>A. versicolor</i> , <i>A. ornatus</i>
	3	<i>A. versicolor</i>
	4	<i>A. flavus</i> , <i>P. jensenii</i>
Coriander	1	<i>A. flavus</i> , <i>M. circinelloides</i> f. <i>griseocyaneus</i>
	2	<i>A. flavus</i> , <i>Absidia repens</i>
	3	<i>A. flavus</i>
	4	<i>A. flavus</i>
Fenugreek powder	1	<i>A. flavus</i> , <i>A. niger</i> , <i>A. ornatus</i> , and <i>P. jensenii</i>
	2	<i>Acremonium</i> sp., <i>P. jensenii</i>
	3	<i>A. versicolor</i>

contaminating smoked and unsmoked Norwegian dry-cured meat products. In their results, total 264 isolates belonging to 20 species and four fungal genera were identified. *Penicillium* sp. constituted 88.3% of the total isolates, and *Penicillium nalgiovense* was the dominant species isolated from both smoked and unsmoked meat products and maintained 38% of the total isolates.

The presence of molds can have either desirable or undesirable results on fermented meat products. Especially, *Penicillium* sp. has been reported to be responsible for commercial covering and the seasoning of sausages and could be used as starter cultures for manufacture of sausages (Iacumin et al., 2009; Leistner, 1986; Sunesen & Stahnke, 2003). Desirable properties of fungi in sausage production are mainly related to the production of secondary metabolites by their lipolytic and proteolytic activity that may increase sensorial flavor profile of the product. On the other hand, undesirable effects of the molds in meat products are usually attributed to capability of producing toxic secondary metabolites such as mycotoxins, with the growth of certain molds (Sunesen & Stahnke, 2003). In the current study, the presence of molds in some Turkish-type fermented sausages (sucuk) samples was investigated. Although mold growth is not desired during production and post-production shelf life of sucuk, sometimes it has been observed and caused marketing problems for consumers. In a related research, it was shown that yeast and mold numbers slightly

increased at the beginning of ripening and then started to decrease with the extending ripening (Genççelep, Kaban, & Kaya, 2007).

Table 4 shows the molds isolated from the meat products and ingredients. In this study, total 55 isolates belonging to *Aspergillus*, *Penicillium*, *Cladosporium*, *Scopulariopsis*, *Paecilomyces*, and *Mucor* sp. were determined in the samples analyzed. The most prevalent genera present in the meat ingredients was *Aspergillus* sp. with the ratio of 94.4%, followed by *Penicillium* sp. Again, 72.2% and 27.8% of *Aspergillus* sp. isolates belonged to *A. flavus* and *A. niger*, respectively. *A. flavus* is known as the major aflatoxin producer.

Spices and herbs are commonly used in food formulations for their aroma and flavor-giving characteristics. However, poor hygienic conditions during their growth, harvesting and drying stages of spice manufacture cause microbial contamination (McKee, 1995). In general, *Aspergillus* sp. forms the mold microflora of spices and herbs (Flannigan & Hui, 1976). For instance, Salari, Najafi, Boroushaki, Mortazavi, and Najafi (2012) examined microbial contamination of 36 Iranian red hot pepper samples collected from Iranian market and found that mold and yeast were generally high ranging from  $2.4 \times 10^3$  to  $4.6 \times 10^6$  cfu/g, and the most predominant fungal genera were *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. Again, Abdel-Hafez, Moharram, and Abdel-Mallek (1987) tested fungal microflora of 40 Egyptian spices consisting of caraway, cumin, fennel, anise, and coriander

**TABLE 5** Aflatoxin levels of meat products analyzed

Sample	Sample ID	Aflatoxin level (ppb)				Total
		AFB1	AFB2	AFG1	AFG2	
Meat	M1	0 <sup>3a</sup>	0	0	0	0
	M2	0	0	0	0	0
Sucuk	S1	0.151	0	0	0	0.151
	S2	0.226	0	0	0	0.226
	S3	0.092	0	0	0	0.092
	S4	0.889	0.067	0	0	0.956
	S5	0.060	0	0	0	0.060
	S6	0.100	0	0	0	0.100
	S7	0.129	0	0	0	0.129
	S8	0.642	0.040	0.077	0	0.759
	S9	0.578	0.027	0	0	0.604
	S10	0	0	0	0	0
	S11	0	0	0	0	0
Sausage	SA1	0	0	0	0	0
	SA2	0.059	0	0	0	0.059
	SA3	0.074	0.020	0	0	0.093
	SA4	0	0	0	0	0
Pastirma	P1	0	0	0	0	0
	P2	0	0	0	0	0
	P3	0.049	0	0	0	0.049
	P4	0	0	0	0	0
	P5	0	0	0	0	0
	P6	0	0	0	0	0
	P7	0	0	0	0	0

<sup>a</sup>Below detection limits.

seeds. The major fungal species found in the spices was *A. fumigatus* while *Emericella nidulans* and *Rhizomucor pusillus* were also observed. All the above results were in accordance with our findings presented in Table 4.

### 3.3 | Aflatoxin contents

Table 5 shows the aflatoxin levels of the meat products collected from Kayseri market. As expected, fresh meat samples did not contain any types of aflatoxin. In the meanwhile, 82% ( $n = 9$ ), 50% ( $n = 2$ ), and 14% ( $n = 1$ ) of sucuk, sausage, and pastirma samples had aflatoxin contents at different levels. AFB1 was the most prevalent type of aflatoxins, and it was observed in 50% of the meat products. It is expected that the presence of aflatoxin in fresh meat occurs only when the animal was fed with contaminated fodders. However, aflatoxin contamination in meat products can be attributed to several other factors such as feeding the animal with aflatoxin-containing rations, incorporation of aflatoxin-contaminated nonmeat ingredients such as cereals and spices as well as growth of toxigenic molds in the outer parts of the meat products (Abd-Elghany & Sallam, 2015). Since pastirma is a salt-cured meat product at high levels, growth of molds is not likely. In this study, total aflatoxin levels of the meat products analyzed were all below 1 ppb (Table 5) which was lower than the findings of Aziz and Youssef (1991) who reported that hot-dog, sausage and luncheon meat in Egypt had aflatoxin levels of 2, 3, and

2 ppb, respectively, while one kubeba sample had a very high aflatoxin level (150 ppb). Sirot, Fremy, and Leblanc (2013) found very low aflatoxin levels (0.05 ppb) of French origin meats, which is similar to our findings. Again, Markov et al. (2013) reported that AFB1 levels of game meats ( $n = 15$ ), semi-dry sausages ( $n = 25$ ) and dry meat products ( $n = 50$ ) were all below 1 ppb. In the study of Refai, Niazi, Aziz, and Khafaga (2003), 40 basterma (a dried cured meat product popularly known in Egypt) samples were analyzed for AFB1 contamination. In the results, basterma samples contained total aflatoxins at levels from 2.8 to 47 ppb. In another study, AFB1 levels of the traditional meat products including hams ( $n = 105$ ), dry fermented sausages ( $n = 208$ ), bacon ( $n = 62$ ), and cooked sausages ( $n = 35$ ) collected from Croatian market were all below the detection limits (Pleadin et al., 2015).

In Table 6, AFB1, AFB2, AFG1, and AFG2 levels of the ingredients, which are commonly used in manufacture of meat products, are presented. As seen in the table, the presence of the aflatoxins was observed in all red paprika, black pepper, and spice mix samples while only 50% and 25% of the coriander and fenugreek samples were exposed to aflatoxin contamination. In this research, a total, 75% of the meat ingredients analyzed contained aflatoxin with the average of 1.808 ppb. According to Turkish Food Codex Regulation of Contaminants (European Commission, 2006; Turkish Food Codex, 2011), maximum AFB1 and total aflatoxin (AFB1 + AFB2 + AFG1 + AFG2) limits for spices including red pepper, black pepper, coriander and their mixtures are approved as 5 and 10 ppb, respectively. These limits are also similar in European Union (EU) regulations (European Commission, 2006). As seen in Table 5, AFB1 and total aflatoxin level of only one red paprika sample (RP2) exceeded the limits while the other samples were in conformity with the EU and Turkish regulations.

Dried herb and spices are commonly contaminated with aflatoxins, which also makes meat products unhealthy if they are used as nonmeat ingredients. Romagnoli, Menna, Gruppioni, and Bergamini (2007) collected 27 aromatic herbs, 28 spices, and 48 herbal infusions and medicinal plants randomly from Italian market in 2000 to 2005 and found that of the 103 samples analyzed and only 7 spices resulted positives: 5 chili-peppers, 1 nut meg, and 1 cinnamon while 2 of them had aflatoxin levels over the permissible limits. Organically produced spices and herbs ( $n = 130$ ) were analyzed by Tosun and Arslan (2013) for the determination of AFB1. In the results, AFB1 levels of 41 organic spice samples were above the EU regulatory limit (5 ppb). Among organic herb samples, the highest concentration of AFB1 (52.5 ppb) was detected in a rosehip sample. AFB1 levels of 21 organic herb samples were above the regulatory limits of the EU. Macdonald and Castle (1996) analyzed total aflatoxin levels of 157 retail spices including curry powders, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon, and coriander while nearly 95% of the samples had total aflatoxin below the limits (10 ppb) determined by EU and only 9 samples had higher levels. In another study conducted by Set and Erkmen (2010), 17.1% (14/82) and 23.1% (19/82) of unpacked ground red pepper samples had total aflatoxin and AFB1 levels exceeding the legal limits, respectively, while only one packed sample had over legal limit of AFB1 by 89.99 ppb. In a similar study, Refai et al. (2003) analyzed total 60 spice samples in terms of AFB1 contamination and determined AFB1 in the spice paste at levels from 9.6 to 120 ppb,

**TABLE 6** Aflatoxin levels of meat ingredients analyzed

Sample	Sample No	Aflatoxin level (ppb)				Total
		AFB1	AFB2	AFG1	AFG2	
Spice mix	SM1	0.184	0.020	0 <sup>4a</sup>	0	0.203
	SM2	0.244	0.020	0	0	0.263
	SM3	0.889	0.040	0	0	0.929
	SM4	0.081	0.023	0	0	0.104
Red pepper	RP1	1.686	0.076	0.133	0	0.896
	RP2	12.848	0.842	0.553	0	14.243
	RP3	1.619	0.118	0.414	0	2.151
	RP4	1.031	0.072	0.058	0	1.161
Black pepper	BP1	0 <sup>4a</sup>	0	0.137	0.046	0.184
	BP2	0.064	0	0	0	0.063
	BP3	0	0	0	4.140	4.140
	BP4	0.042	0	0.343	1.192	0.578
Coriander	C1	0	0	0	0	0
	C2	0.067	0.019	0	0	0.086
	C3	0	0	0	0	0
	C4	0.050	0	0	0	0.050
Fenugreek powder	FF1	0	0	0	0	0
	FF2	0	0	0	0	0
	FF3	0	0	0	0	0
	FF4	1.009	0.056	0	0	1.065

<sup>a</sup>Below detection limits.

which were in pepper (285.6 ppb), garlic (224.4 ppb), fenugreek (194.2 ppb), coriander (166.4 ppb), and capsicum (42.4 ppb).

## 4 | CONCLUSION

In this study, total 46 samples comprised of different meat products (fresh meat, sucuk, pastirma, and sausage) and their ingredients (black pepper, red paprika, spice mix, coriander, and fenugreek powder) were analyzed for their fungi microflora and aflatoxin contents. About 27% and 43% of sucuk ( $n = 3$ ) and pastirma ( $n = 3$ ) samples had the mold contents over the limits established by Turkish Food Codex, respectively. Total 55 isolates belonging to *Aspergillus*, *Penicillium*, *Cladosporium*, *Scopulariopsis*, *Paecilomyces*, and *Mucor* genera were determined in the meat products and the ingredients analyzed while the most prevalent genera present in the meat ingredients was *Aspergillus* sp. with the ratio of 94.4%, followed by *Penicillium* sp. 72.2%. In the case of aflatoxin analyses, sucuk, sausage, and pastirma samples had total aflatoxin levels below 1 ppb. On the other hand, aflatoxin levels (14.243 ppb) of 25% of red pepper samples exceeded the limits established by both Turkish Food Codex and European Commission regulations. HPLC method was successfully used for the determination of aflatoxins. In conclusion, this study showed that more careful hygiene applications should be performed in order to minimize mold and aflatoxin contamination of the meat products and their ingredients produced in food industry in Turkey.

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## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## ORCID

Hasan Yetim  <http://orcid.org/0000-0002-5388-5856>

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