

## Aflatoxin in Spices Marketed in the West Mediterrenian Region of Turkey

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**Abstract:** Aflatoxins display an insufficiently recognized risk to human health. They can be found in a variety of food items and are not destroyed by normal industrial processing or cooking. In this study, totally 134 spice samples including powdered red pepper, flaked red pepper, black pepper and cumin were investigated for total aflatoxin levels by ELISA. Spice samples were obtained from bazaars and markets in Burdur, Isparta and Antalya city centers. Total aflatoxin values of the samples not exceeded  $10 \mu\text{g kg}^{-1}$  as legal limit of the European Union. All of the red pepper samples were contaminated with levels ranging from  $3.55\text{-}9.55 \mu\text{g kg}^{-1}$ . Black pepper samples contained levels ranging from  $0.67\text{-}6.15 \mu\text{g kg}^{-1}$ . Cumin aflatoxin levels were found to be ranging from  $4.55\text{-}8.57 \mu\text{g kg}^{-1}$  while flaked red pepper contained aflatoxin levels maximum  $9.46 \mu\text{g kg}^{-1}$ .

**Key words:** Aflatoxin, spice, West Mediterrenian, human health, cooking, Turkey

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### INTRODUCTION

Aflatoxins display an insufficiently recognized risk to human health. They are formed in stored nuts, cereals and rice under high humidity and temperature conditions (Peraica *et al.*, 1999). They can be found in a variety of food items and since they are heat-stable, they can not be destroyed by normal industrial processing or cooking (MacDonald and Castle, 1996; Creepy, 2002).

Aflatoxins are a group of closely related heterocyclic compounds produced predominantly by two filamentous fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Recent studies have shown that some *A. nomius* and *A. tamaris* strains are also produce aflatoxin (Mishra and Das, 2003). Ito *et al.* (2001) isolated one more strain, *A. pseudotamaris* that can produce aflatoxin.

Aflatoxins contaminate a vast array of food and agricultural commodities. *Aspergillus* species are capable of growing on a variety of substrates and under a variety of environmental conditions.

Therefore, most foods are susceptible to aflatoxigenic fungi at some stage of production, processing, transportation and storage (Mishra and Das, 2003). Presently, 18 different types of aflatoxins have been identified with aflatoxin B1, B2, G1, G2, M1 and M2 being the most common. Of these, Aflatoxin B1 (AFB1) and G1 (AFG1) occur most frequently. Biological effects of aflatoxin can be subdivided into its toxicity,

carcinogenicity, mutagenicity and teratogenicity. The effects are influenced by species variation, sex, age, nutritional status and effect of other chemicals. In addition, the dose level and period of exposure of the organism to the toxin are very important (Mishra and Das, 2003).

AFB1 is the most abundant and toxic form of all naturally occurring aflatoxins. AFB1 represents 75% of all aflatoxins found in contaminated food and feeds. It is hepatotoxic, hepa-tocarcinogenic and teratogenic to various animal species. The formation of AFB1-DNA adduct is highly correlated to the carcinogenic effect of AFB1 in both animal and human cancer cases (Ayub and Sachan, 1997).

Aflatoxicosis characterized by jaundice, ascites, portal hypertension and other signs of hepatic failure has been described in humans exposed to 2-6 mg of aflatoxin daily for approximately 1 month after consumption mold damaged-corn. Immunosuppression caused by aflatoxins has been demonstrated in laboratory animals, although virtually no data are available on the immunosuppressive effects of aflatoxins in human populations (Eaton and Gallagher, 1994).

In this respect, aflatoxin contamination in spices is important for the public health. In current study, total aflatoxin levels in spices was detected (red pepper, black pepper, cumin and flaked red pepper) that were obtained from West Mediterrenian region of Turkey.

## MATERIALS AND METHODS

**Samples:** A total of 134 spice samples that were obtained from bazaars and markets in Burdur, Isparta and Antalya city centers were evaluated in the experiments. These spices were black pepper, powdered red pepper, cumin and flaked red pepper.

**Aflatoxin assays:** Helica Biosystems MycoMonitor Total Aflatoxin ELISA kit (Helica Biosystems Inc. Fullerton, CA, USA) was used for quantitative detection of total aflatoxin in the samples following manufacturer's instructions. The microwells were measured at 450 nm by an ELISA reader. The optical densities of the samples were compared with the OD's of the kit standarts and an interpretative result was determined.

Spice samples were exposed to some pretreatments. About 20 g of the spice sample was mixed with 100 mL of 70% methanol in a beaker for 2 min. Mix was filtrated by a filter paper. This filtrate were used in the above mentioned assay.

## RESULTS AND DISCUSSION

Total Aflatoxin levels of the spices that evaluated in this study were shown in Table 1. Total aflatoxin values of the samples not exceeded 10  $\mu\text{g kg}^{-1}$  as legal limit of the European Union. All of the red pepper samples were contaminated with levels ranging from 3.55-9.55  $\mu\text{g kg}^{-1}$ . Black pepper samples contained levels ranging from 0.67-6.15  $\mu\text{g kg}^{-1}$ . Cumin aflatoxin levels were found to be ranging from 4.55-8.57  $\mu\text{g kg}^{-1}$  while flaked red pepper contained aflatoxin levels maximum 9.46  $\mu\text{g kg}^{-1}$ . Insufficient hygiene conditions during drying, transport and storage stages in the production of red pepper could cause microbiological and mycological growth which could result in the formation of mycotoxins (Aydin *et al.*, 2007). In a similar study in ground deep-red pepper samples from Sanliurfa (Turkey), 72 of the 75 samples (96%) contained AFB1 in the range of 0.11-24.7  $\mu\text{g kg}^{-1}$ . Eleven (14.7%) samples were above the regulatory limits used in the European Union and in Turkey (Ardic *et al.*, 2008). Aydin *et al.* (2007) evaluated Aflatoxin B<sub>1</sub> levels in 100 red pepper samples that randomly obtained from Istanbul markets.

Table 1: Total aflatoxin levels of the spices

Samples	No. of samples (n)	Aflatoxin levels		
		mean±SE ( $\mu\text{g kg}^{-1}$ )	Min.	Max.
Red pepper	34	6.36±0.56	3.55	9.55
Black pepper	34	3.44±0.38	0.67	6.15
Cumin	28	7.03±0.28	4.55	8.57
Flaked red pepper	38	4.79±0.72	ND	9.46
Total	134	5.31±0.32	ND	9.55

According to the results, values of 32 samples were below the minimum detection limit in 50 samples were between 0.025 and 5  $\mu\text{g kg}^{-1}$  and 18 samples had unacceptable contamination levels higher than the maximum tolerable limit (5  $\mu\text{g kg}^{-1}$ ) (Aydin *et al.*, 2007). Bircan (2005) evaluated 75 spice samples marketed in Turkey. According to results, 27 paprika all the chilli powder and four ground black pepper samples were contaminated with aflatoxin B<sub>1</sub> in the range of 0.5-116.4, 1.6-80.4 and 0.3-1.2  $\mu\text{g kg}^{-1}$ , respectively. About 23 paprika and chilli powder samples were above the regulatory limits used in the European Union. Aflatoxin contamination was not detected in the cumin samples (Bircan, 2005).

Colak *et al.* (2006) were randomly obtained 84 samples of spices (30 red-scaled pepper, 30 red pepper and 24 black pepper) from Istanbul markets and bazaars. They reported that 17 out of 30 red-scaled pepper samples (56.7%) contained total aflatoxins ranging from 0.7-46.8  $\mu\text{g kg}^{-1}$  while 13 out of 30 samples contained AFB1 ranging from 1.9-35.5  $\mu\text{g kg}^{-1}$ . In red pepper, 11 out of 30 samples (36.7%) contained total aflatoxins ranging from 0.8-15.4  $\mu\text{g kg}^{-1}$ . The concentrations of AFB1 were found to be ranging from 2.9-11.2  $\mu\text{g kg}^{-1}$ .

The levels of total aflatoxins were determined to be ranging from 0.3-16.7  $\mu\text{g kg}^{-1}$  in 8 out of 24 black pepper samples. Only two samples contained AFB1 at the levels of 9.8 and 10.3  $\mu\text{g kg}^{-1}$  (Colak *et al.*, 2006). Erdogan analysed 44 red-scaled pepper and 26 red powder pepper samples obtained from various spice retailers in Erzurum and 20 isot (red pepper produced in Sanliurfa) were investigated for aflatoxin contamination using thin layer chromatography. Aflatoxin (B+G) was found in 8 red pepper samples (18.2%) and 3 red powder peppers (10.7%) and 1 isot sample (5%).

The aflatoxin amount ranged from 1.1-97.5 ppb in all samples investigated. The highest amount of aflatoxin was found in red-scaled pepper (Erdogan, 2004). However, aflatoxin levels of spices in Europe are varied in different countries. In a study that achieved in Lisbon (Portugal), 79 prepackaged samples of 12 different types of spice powders (5 cardamom, 5 cayenne pepper, 8 chilli, 5 cloves, 7 cumin, 5 curry powder, 5 ginger, 5 mustard, 10 nutmeg, 12 paprika, 5saffron and 7 white pepper) were analysed. AFB1 was detected in 34 samples of prepackaged spices (43.0%). All of the cayenne pepper samples were contaminated with levels ranging from 2-32  $\mu\text{g kg}^{-1}$ . About 3 nutmeg samples contained levels ranging from 1-5  $\mu\text{g kg}^{-1}$ , 3 samples had levels ranging from 6-20  $\mu\text{g kg}^{-1}$  and there were two with 54 and 58  $\mu\text{g kg}^{-1}$ . Paprika contained levels of aflatoxin B1 ranging from 1-20  $\mu\text{g kg}^{-1}$ . Chilli, cumin, curry powder, saffron and white pepper samples had levels ranging from 1-5  $\mu\text{g kg}^{-1}$ .

Aflatoxins were not detected in cardamom, cloves, ginger and mustard (Martins *et al.*, 2001). Romagnoli *et al.* (2007) reported that only 7 of 103 spices that collected from Emilia Romagna Region, Italy were aflatoxin positive.

### CONCLUSION

Aflatoxins can be found in a variety of food items and since, they are heat-stable, they can not be destroyed by normal industrial processing or cooking (Creepy, 2002; MacDonald and Castle, 1996). Hygiene and storage conditions should be ameliorated for the prevention of aflatoxin contamination in spices. According to Mishra and Das (2003) most rational and economic approach is preharvest elimination of aflatoxin. Prevention of aflatoxigenic fungal attack is possible by using healthy seeds, proper irrigation, rotation of crops, harvesting after full maturity, drying the harvested crops within time and store them under proper atmosphere, preventing insect or other damages to the crop (Mishra and Das, 2003). However, according to some researchs, ozone application may presents a solution for aflatoxin contamination problem (Inan *et al.*, 2007; Akbas and Ozdemir, 2006).

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