

Monitoring aflatoxin contamination risks in red pepper spice produced in Turkey

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Abstract

Even though Turkey is the third leading producer of fresh pepper, currently it only supplies 3-5% of processed red pepper spice in the international trade market because of high aflatoxin contamination. This study was designed to determine the presence and levels of aflatoxins in red pepper spice collected from three major producer provinces in Southeast (K. Maras, G. Antep, Hatay-Kilis) and one province in Central Turkey (Ankara). This pilot study also included companies that are actively involved in international red pepper spice trade. Nearly, half of the samples showed significant levels of contamination above the European Standard of 5 ng/g for AFB₁ and 10 ng/g for total aflatoxins. Over ninety percent of contaminated samples contained AFB₁ with levels ranging from 5 to 85.55 ng/g. The overall mean level of total aflatoxins was 22.18 ng/g with the highest level being 144.41 ng/g. These results indicate that there is a necessity for establishing stricter and continuous national surveillance schemes for improving safety and quality of red pepper spice in Turkey.

Key words: *Aspergillus*, aflatoxin, red pepper, food safety

Introduction

Pepper (*Capsicum annuum*) is an important agricultural crop in Turkey with annual production exceeding 1 million tonne (fresh weight) per year (Abak, 1994). Annually 80,000-100,000 tonnes of fresh pepper is processed into 20,000-25,000 tonnes red pepper spice (dry), an important commercial commodity in local trade and the national export economy (Kahramanmaraş Commerce Chamber, 2006). Red spice pepper is a natural food additive, a key flavor and distinctive color component in processing of ready-to-eat meat products, sausages, and some fish dishes, also an important ingredient in spice mix formulations. It has also been used for centuries in folk medicine

for its therapeutic properties of reducing abdominal pain, stomach ulcers, diarrhea, and common cold. Also, pepper is used as appetite stimulator. Recently it has been recorded that some chemical components in pepper show beneficial health effects of antioxidative, antimutagenesis and antitumorigenesis (e.g., capsaicin) (Rosa et al., 2003; Zhang et al., 2003), and in vitro studies demonstrated that hot pepper prevents lipid peroxidation in brain cells (Oboh et al., 2007).

Red pepper spice in Turkey is produced predominantly in hot and humid climate of Southeast Region of Turkey (G. Antep, K. Maras, Urfa, Kilis, and Hatay). In the province of K. Maras alone, over 100 production facilities produce 10.000 tonnes/year red pepper spices, which constitute nearly 45% of the total national production. In the old traditional production approach, peppers are cut and seeds are removed. Cut-peppers are then spread in a single layer on soil or concrete ground, and sun-dried under unhygienic conditions. The surface of peppers can suffer mechanical damage in the field and as a result fungal invasion occurs with or without visible symptoms. As a consequence, high aflatoxin (AF) contamination is difficult to avoid. In recently adopted more modern methods including the steps of, (1) low quality damaged, blemished or spoiled fresh peppers are removed by hand-picking on conveyor belts before the drying process. (2) The whole seed-bearing fruits are then washed in pools and then in spray showers to remove soil particles. (3) After that peppers are cut/sliced and carried into the dehydrator where hot air and indirect steam is applied at 60 ± 10 °C to lower the final moisture to 12%. The dried peppers are finely ground and then vegetable oil is added.

Although adulteration of red pepper spice with carotene rich tomato peel and/or synthetic Sudan azo dyes (illegal) to enhance appearance has been reported by national news agencies, the principal problem of red pepper spice is the high level of AF contamination (M.A. Report, 2008). AF production by toxigenic *Aspergillus* spp. may occur before harvesting, or alternatively AF producing strains can remain or recontaminate during or after processing with toxins produced during storage. It is very difficult, if possible, to remove AFs since they are heat-resistant and only partially soluble in polar solvents. Considering the persistence in the human food chain, and the toxicological and health significance; contaminant levels must be monitored from 'farm to fork' with particular attention to red pepper spice use in ready-to-eat foods which are not subjected to further heat treatments prior to consumption.

In 1996, AFs contamination of red pepper spice produced in Turkey came to international attention following reports of large amounts of AFs in spice peppers marketed in Germany that resulted in a European trade barrier for

nearly 9 years during which spice pepper industry suffered from huge economic and devastating image loss (Milliyet, 2005). Subsequently, the Turkish Food Codex changed the AF contamination limits to be comparable to the European Union members, its major trade partners. The objective of the pilot survey was to test for the presence and concentration of AFs in processed ready-to-use red pepper spice produced in several provinces of Turkey. Three of these districts constitute nearly 90% of the red pepper spice production. This pilot study also afforded the opportunity to include companies that are actively involved in international trade. One of the goals in this pilot analytical survey was to respond to the strong health concerns of the general public about the high AF contamination risks of red pepper spice that has generated recent public anxiety due to bad publicity in news bulletins.

Materials and Methods

Sample preparation

Fifty-one samples of red pepper spice were randomly collected from producers, distributors to local spice stores, public markets, and production facilities in G. Antep, K. Maras, Hatay-Kilis, and Ankara. Fifteen of these samples were obtained from production facilities of companies that engage in international trade of spice pepper. Samples were stored at -20 °C until analysis.

Extraction of AFs by Immunoaffinity Column and HPLC analysis

A slightly modified version of the Official Method of Analysis (AOAC) for determination of AF content (AFB₁, AFB₂, AFG₁, and AFG₂) in nut products (Basaran and Ozcan, 2009) was used for determination of AFs in spice red pepper. AFs were extracted with methanol and bound on immunoaffinity column (AflaPrep, R-Biopharm Rhone Ltd., Scotland). The AFs were then eluted from the column by applying 250 µl of methanol:dH₂O (1:2) (Basaran and Ozcan, 2009). AF identification was carried out by means of a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan), a SIL-20AC Prominence auto sampler, a LC-20AT Prominence pump, and a Tracel Extracil ODS2 reverse column (5µ, 3.5 µm, 24 × 0.46 mm). A post-column derivatisation was applied to produce electrochemically generated bromine (Kobra Cell, R-Biopharm Rhone Ltd., Scotland) (Basaran and Ozcan, 2009).

Results and Discussion

Fifty-one samples of red pepper spice from randomly selected stores in three provinces of Southeast Turkey and the Central province of Ankara were analyzed for the incidence AFs (AFB₁, AFB₂, AFG₁, and AFG₂) contamination. The distribution of the number of positive samples for the different provinces is summarized in Table 1. Out of fifty one of samples, twenty showed potentially significant levels of AFB₁ (5 ng/g) and total AF (10 ng/g) contamination above the European Standard. The overall mean total concentration of AFs in 51 samples was 22.18 ng/g with the highest level being 144.41 ng/g. The predominant AF was AFB₁. 92% (45/51) spice samples contained AFB₁ with mean levels of 17.3 ng/g. G. Antep had significantly lower levels of total AF and 2 out of 12 samples were positive (Figure 1). In K. Maras, seven samples out of thirteen of red pepper spice samples tested were contaminated; 6 of the 7 were contaminated with AFB₁ and levels of contamination were moderately lower than in other provinces surveyed in the study (Table 1). Although, the percentage of contamination in samples collected from Hatay-Kilis was comparable to K. Maras, the level of AFB₁ contamination was much higher (2.49–32.54 ng/g). Data for Ankara, where no red pepper production occurs and all the red peppers are brought in from Southeast of Turkey and stored until consumption, indicated that 10 of 12 samples (92%) contaminated with (contained-remove) AFB₁ at a concentration of >5 ng/g, with a maximum of 96.9 ng/g.

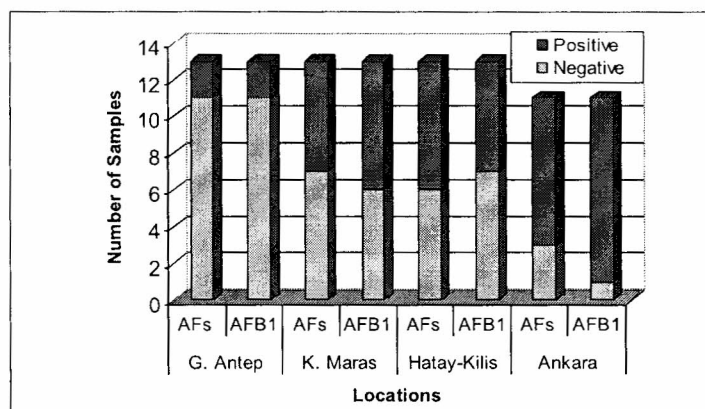


Figure 1. Occurance of AFs in spice red peppers exceeding national limits in four provinces in Turkey

The region of Southeast Anatolia (G. Antep, K. Maras, and Hatay-Kilis) is a zone in which red pepper has traditionally been cultivated for many decades and where a number of private investors produce hot paprika for local and export markets. The summer temperature in the region may reach as high as 43°C, and red pepper spice finds a place in the local diet as respiration stimulant to lower body temperature. The combined high humidity and moderate temperature in the rest of the year favors fungal growth of toxic *Aspergillus* spp. When samples from G. Antep and K. Maras

were compared for conventional open air and integrated air driers production facilities, none of the 8 samples in K. Maras and only one of 7 samples in G. Antep obtained from red pepper spice production facilities with integrated dryers and integrated traceability programs contained AFs above the permitted limits.

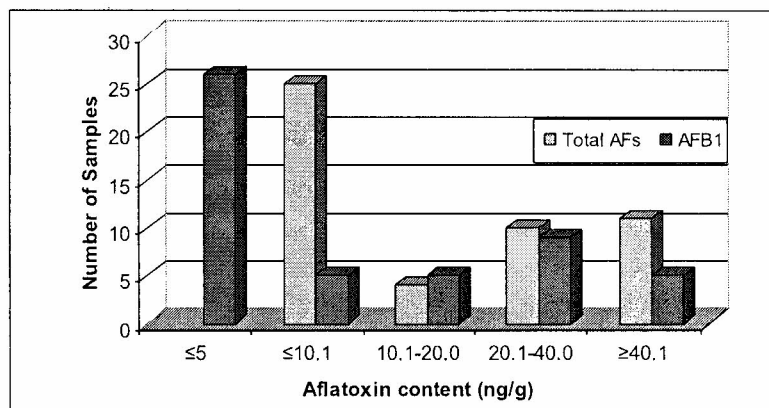


Figure 2. Distribution of AF quantities in samples collected from four different provinces in Turkey

Figure 2 summarizes the distribution of AFs based on concentration. Twenty-six of 51 samples, (>50%) were not contaminated with AFB₁ concentrations greater than 5 ng/g, the maximum tolerated regulatory limit, 5 samples (10%) contained > 10 ng/g, and 14 samples (27%) >20 ng/g. The results indicated that out of 51 samples, 25 (49%) were contaminated with total AFs at or above legal limit of 10 ng/g given by European Commission (Figure 2). A number of earlier surveys in Turkey indicated the chances of AF contamination in red pepper spice were widespread. The first published report of AFs in red pepper spice comes from Bursa and Sakarya, North-West provinces (Yildirim et al., 1997). Eight of 34 samples (23.5%) were contaminated with AF at concentrations >5 ng/g with a maximum of 15 ng/g (Yildirim et al., 1997). In 1998, Hazir and Coksoyler found 46 of 141 red pepper spice samples taken from several provinces were AF contaminated. Later Erdogan (2004) found 8 of 44 red pepper spice samples obtained from the province of Erzurum were contaminated at levels ranging from 1.1 to 97.5 ng/g. Bircan (2005) detected contamination more frequently in red pepper spice than in other spices surveyed and all 27 samples obtained from Western Turkey were contaminated with AFB₁ at levels ranging from 0.5 to 116.4 ng/g. Data for the province of Sanliurfa (Ardic et al., 2008), indicated that 72 of 75 red pepper spice contained AFB₁ and 11 of which were above the limits set by the Turkish Codex with a maximum of 24.7 ng/g. The most recent survey carried out by the Turkish Ministry of Agriculture revealed that 44 out of 472 red pepper spice samples, were contaminated with AFB₁ at or above 5 ng/g (M.A. Report, 2008).

The use of herbs and spices is increasing in the food industry to enhance flavor and aroma, especially in ready-to-eat (RTE) meals, and exotic dishes with improved functionalities. This trend increases the public concern in regards to potential contaminants in herbs and spices. Numerous recent reports identified AFs in spices and, in particular, red pepper. Reddy et al. (2001) reported an AF contamination problem in India, the major chili pepper producer worldwide. Of 182 chili samples, more than 59% were contaminated with AFB₁ concentrations >5 ng/g, and 9% of samples contained >30 ng/g, with a maximum total AF of 969 ng/g. Paterson (2006) reported 100% incidence of contamination of AFs in 13 samples obtained from Pakistan, with a range of contamination of total AFs in chili samples was 0.1–96.2 ng/g with mean concentrations of 32.11 ng/g of AFB₁. An AF survey of hot pepper spice in Italy in 2006 showed that while nearly half of all samples contained AFs, and about 20% contained AFs at non-permissible levels (Romagnoli et al., 2006). The data for the USA (Wood, 1989), demonstrated that 9 of 12 red pepper samples (75%) contained AFB₁ at a concentration >5 ng/g with a maximum of 30 ng/g. The incidence of AFs contamination in chili powder surveyed in UK was over 50%, and the maximum detected contamination level was 61 ng/g (Patel et al., 1996). O’riordan and Wilkinson (2007) surveyed AFs in spices imported and sold in Ireland and detected the highest concentration of total AF was 27.5 ng/g.

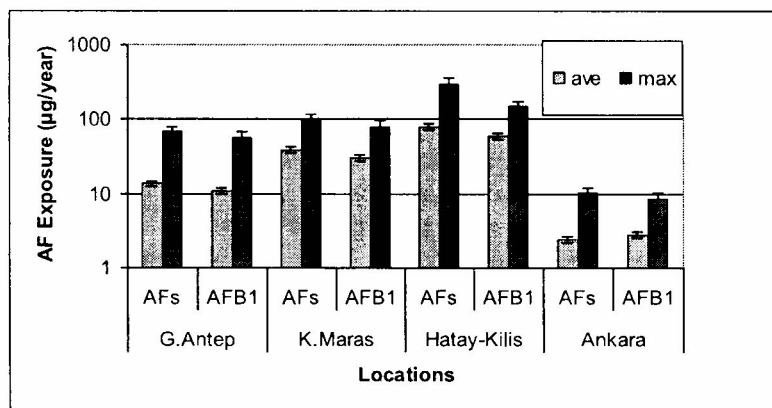


Figure 3. Estimated average (ave) and maximum (max) consumer exposure (µg/year) to AFs within four selected four province

Table 1. Analytical results (in ng/g) of red pepper spice samples collected from four provinces in Turkey

Province	Number of samples	AFB1			AFB2		AFG1				AFG2		
		Positive samples Highest Mean±SD			Positive samples Highest Mean±SD		Positive samples Highest Mean±SD				Positive samples Highest Mean±SD		
G. Antep	13	13	23.03	4.3±1.09	10	2.71	0.49±0.1	4	4.85	0.61±0.09	3	0.13	0.01±0.01
K. Maras	13	13	33.8	12.6±1.92	12	3.53	1.11±0.1	13	7.2	2.43±0.06	0	0	0±0
Hatay-Kilis	13	13	96.9	23.9±4.03	13	11.1	2.3±0.2	13	34.81	5.93±1.54	3	1.57	0.5±0.01
Ankara	12	11	85.55	27.12±3.9	9	18.1	2.02±0.2	9	1.96	0.28±0.08	9	15.14	2.13±0.02

Considering the low amount of consumption, it is after assumed that spices are not a relevant contributor to the AF exposure in the human population. However, the use of large quantities in some cultures may present high health risks. A small survey of in Turkey (data not shown) revealed that the average household consumption of red pepper spice is 2.5 kg/year/person in the Southeast provinces and 50 g/year/person for those living in Ankara. Taking into account the average consumption of red pepper in Southeast Turkey and the estimated mean as well as the highest level of total AFs and AFB₁ intakes ($\mu\text{g}/\text{year}$) an estimate AF exposure on a yearly basis was calculated (Figure 3). The population in Hatay-Kilis was exposed to maximum levels of AFB₁ and total AFs of 147 $\mu\text{g}/\text{year}$ and 361 $\mu\text{g}/\text{year}$, respectively (Figure 3). The exposure to AFs in the Southeast provinces of Turkey was 10 times higher than that of consumers living in Ankara. Based on the surveillance data, the average consumers in Hatay-Kilis were exposed to AFs (80.90 $\mu\text{g}/\text{year}$) more than twice those purchasing the product from K. Maras (37.47 $\mu\text{g}/\text{year}$). As red pepper spice is consumed all year around, it might result in accumulation in the body. According to the Joint Expert Committee on Food and Additives (JECFA) recommendations, the tolerable weekly intake (TWI) of ochratoxin a week fungal toxin is 100 ng/kg body weight (JECFA, 1995). As AFs are highly toxic, current recommended levels are "as low as possible" (Herrman and Walker, 1999).

Contamination of food commodities with AFs is significant not only in terms of food safety and public health but also for even international trade economics. Ordinarily, authoritarian precautions are influenced greatly by economic considerations at national levels but even more effectively at an international level. Food safety enforcement and surveillance is regularly carried out by the Public Analysis Laboratories of Turkish Ministry of Agriculture. If the product is anticipated to be exported domestic as well as internationally accredited laboratories test and certify the quality of the product. Further official AF analyses are carried out for confirmation only if a dispute between traders and regulatory enforcement authorities exists and a case carried to the court (Milliyet, 2005).

Primary opportunities of *Aspergillus* contamination are during hand-picking, open air sun drying on unprotected soil and, the storage unsuitable and uncontrolled facilities. Prevention approaches including pre-selection of high-quality undamaged fresh pepper and appropriate drying method, could greatly enhance the keeping quality of final product. In recent years, several novel methods including ionizing radiation, steam, hot air circulating tunnel dryers, solar tunnel dryers, infrared heating and microwave applicators have been tested for cost-effectiveness in red pepper spice treatment (Hossaina et al., 2005; Kispetera, et al., 2003; Staack et al., 2008). Some approaches have merit but further research and development activities are warranted to test their effectiveness for controlling AF contamination in red pepper spice in Turkey.

This project was one of the most comprehensive surveys for red pepper spice undertaken in geographically variable regions in Turkey. Based on the results in this study, there is a scientific rationale for establishing stricter and continuous national

surveillance schemes for red pepper spice. Beyond food sanitary objectives, the measures that will be taken by national authorities would contribute strongly to keeping the positive image of producers, and especially exporters, for promoting, and increasing economic competitiveness of Turkish red pepper spice in international markets.

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