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Determination of Aflatoxin B_1 levels in powdered red pepper

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Abstract

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. This study was designed to assess the aflatoxin B_1 levels in 100 samples of powdered red pepper randomly obtained from markets in Istanbul using microtitre plate Enzyme Linked Immunosorbent Assay (ELISA). Aflatoxin B_1 levels were below the minimum detection limit (0.025 μg/kg) in 32 samples, between 0.025 and 5 μg/kg in 50 samples, whilst 18 samples had unacceptable contamination levels higher than the maximum tolerable limit (5 μg/kg), according to the Turkish Food Codex and the European Commission.

Keywords: Aflatoxin B_1; Powdered red pepper; Traditional food; ELISA

1. Introduction

Most spices are produced in tropical and subtropical regions. The hot and humid climate, production conditions with extended drying times, and often-inadequate instructions to the farmers, may cause considerable quality problems (Gerhard, 1994). It has been reported that 5–10% of agricultural products in the world are being spoiled by mould to the extent that they cannot be consumed by human and animals (Topal, 1993).

Mould growth in agricultural products may cause an important hazard to human health by the formation of toxic metabolites called "mycotoxin". Aflatoxins (AF) belong to the group of mycotoxins. AF are a group of highly toxic secondary metabolic products of *Aspergillus flavus*, *A. parasiticus* and have carcinogenic, teratogenic effect to livestock and humans (Piva, Galvano, Pietri, & Piva, 1995).

Red (chilli) peppers are native for Central and South America. Portuguese traders introduced them to Europe, Middle East, India, Indonesia and other parts of Asia around 450–500 years ago (Berke, 2002). The world's largest producer of red (chilli) peppers was China at 8.1 million metric tons in 2000. Turkey is one of the major red pepper producing countries together with India, Mexico, Spain and USA (FAO, 2000; Times Agricultural Journal, 2002). Red pepper is sensitive to aflatoxin contamination depending on atmospheric temperature, humidity, drying and processing conditions (Çoksöyler, 1999).

Powdered red pepper is one of the favourite spices in Turkey and is commonly used for flavouring, seasoning and imparting aroma or colouring of foods. Therefore, the aim of this study was to determine the Aflatoxin B_1 (AFB_1) levels in powdered red pepper, in the respect of maximum tolerable limits in Turkish Food Codex and European Commission and to indicate the importance of contamination regarding the public health. This study was conducted

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by ELISA method of which has been used first in Turkey for powdered red pepper analyses.

2. Materials and methods

2.1. Material

A total 100 powdered red pepper samples obtained randomly from retail sales points in Istanbul were analyzed during April and May 2004. 50 samples in April and 50 samples in May have been collected into sterile jars from different sales points. 100 g of each sample have been immediately brought to the laboratory at 4–6 °C and analyzed. Microtitre plate enzyme-linked immunosorbent assay (ELISA) reader (ELX 800, Bio-tek Inst.) and AFB_1 test kit (Ridascreen, R-biopharm, Germany) were used to run ELISA analyses.

2.2. Method

The quantitative analysis of AFB_1 levels were determined in the powdered red pepper samples. The samples were analyzed using the AFB_1 test procedure (Art. no: R1201), which was described by the producer company (r-biopharm) (Enzyme Immunoassay for the quantitative analysis of aflatoxins, 1999).

2.2.1. Preparation of samples

Hundred gram powdered red pepper sample mixed in aseptic conditions in the jars and 10 g of red pepper sample has been sub-sampled 50 ml of 70% methanol (35 ml methanol, 15 ml distilled water) were mixed by magnetic stirrer (Janke & Kunkel, Germany) for 10 min. The extract was filtered (Whatman no. 1), to 5 ml of the filtered solution, was 15 ml distilled water, then 0.25 ml Tween 20 (Merck, 8.22184) was added. The solution was mixed by magnetic stirrer for 2 min.

2.2.2. Separation with Aflatoxin column

Rida® Aflatoxin column (R-biopharm GmbH., Art No: R 5002) was used for AFB_1 R-biopharm GmbH test procedure. The column was rinsed with 2 ml distilled water for equilibration. The column was filled with approximately 1 ml sample extract. A suitable adapter was attached on top of the column and a syringe was used as a sample reservoir. Syringe was filled with the rest of the sample extract. This was passed slowly and continuously through the column (flow rate: approx. 1 drop/s) and discarded. The column was rinsed with 10 ml distilled water and the passed solution was discarded. The column was dried by passing air through the column for approx. 10 s, in order to make sure that all the residual buffer would be removed from the column. The syringe was removed and a clean and closable vial directly below the column. 5 ml methanol was passed slowly through the column (flow rate: approx. 1 drop/s).

2.2.3. Aflatoxin B_1 analysis

Toxin containing eluate was diluted (1 + 9) 1:10 with the corresponding sample dilution buffer (Phosphate Buffer Solution (PBS), pH 7.2) of the respective test (50 μl + 450 μl sample dilution buffer). 50 μl aflatoxin standard solutions and 50 μl prepared test samples were added into separate wells of micro-titer plate, in duplicate. Plates were incubated for 2 h at room temperature in the dark. The liquid was then removed completely from the wells, the each well was washed with 250 μl washing buffer (PBS-Tween-Buffer, pH 7.2) and this was repeated two more times. Subsequently, enzyme substrate (urea peroxide, 50 μl) and chromogen (tetramethyl-benzidine, 50 μl) were added to each well and incubated for 30 min at room temperature in the dark. 100 μl of the stop reagent (1 M H_2SO_4) was added and the absorbance was measured at 450 nm in ELISA reader.

2.2.4. Evaluation

The mean values of the absorbances for the standards and the samples were evaluated according to the Rida® Soft Win program (RIDAVIN.EXE) distributed by Ridascreen (R-Biopharm). The detection limit of the AFB_1 test in the analytical procedure was 0.025 μg/kg, recovery rate was 50–70% and the average coefficient of variation was 8% (Enzyme Immunoassay for the quantitative analysis of aflatoxins, 1999).

3. Results and discussion

Powdered red pepper is amongst the most consumed spices in the world (Ahmad & Ahmed, 1995). A number of studies have been held on hygienic quality of red pepper throughout the world (Table 1).

AFB_1 levels in 68 (68%) of 100 powdered red pepper samples were higher than the detection limit (0.025 μg/kg). AFB_1 levels were found to be higher than the legal limits of Turkish Food Codex (2002) and European Commission (2002) (>5 μg/kg) in 18 (18%) red pepper samples (Table 2). The highest AFB_1 level in the samples was 40.9 μg/kg.

Wood (1989) found that 75% of 12 red pepper samples were contaminated with AFB_1 with a maximum aflatoxin level of 30 μg/kg in USA. Ahmad and Ahmed (1995), analyzed 176 red pepper samples in Pakistan and 66% of samples were found to be contaminated with AFB_1. Although the authors found the AF levels generally lower, however average AFB_1 levels were found 25 μg/kg in seven red pepper samples.

Reddy, Mayi, Reddy, Thirumala-Devi, and Reddy (2001) tested of 182 chilli samples and 59% of the samples were contaminated with AFB_1. Maximum AFB_1 level was 969 μg/kg in chilli pepper grade 3 samples. The authors reported that 40% of the chilli powders, sold in supermarkets, contained aflatoxins and 9% of them contained aflatoxin >30 μg/kg.

Although detected AFB_1 percentages were similar to the results of Wood (1989), Martins et al. (2001), FSAI (2004) and Zinedine et al. (2006), AFB_1 percentages were higher

Table 1
The occurrence of AFB_1 in red pepper reported in previous studies

References	Country	Pepper variety	Number of Sample	Positive (%)	Range (μg/kg)
Wood (1989)	USA	Red pepper	12	9 (75.0%)	ND-30[a]
Ahmad and Ahmed (1995)	Pakistan	Red pepper	176	117 (66.0%)	ND-25
Fufa and Urga (1996)	Ethiopia	Ground red pepper	64	8 (12.5%)	250–525
Yıldırım et al. (1997)	Turkey (Bursa, Sakarya)	Red pepper	34	8 (23.5%)	1.6–15
Hazır and Çoksöyler (1998)	Turkey (Kahramanmaraş, Gaziantep)	Red pepper	141	46 (32.6%)	0.45–80.25
Karagöz (1999)	Turkey (Ankara)	Powdered red pepper	25	20 (80.0%)	1.3–19.8
		Red-scaled pepper	25	23 (92.0%)	1.2–14.8
Çoksöyler (1999)	Turkey (Kahramanmaraş)	Pepper	9	4 (44.4%)	20–80
Reddy et al. (2001)	India	Chilli grade 1	42	21 (50.0%)	<10 – 100
		Chilli grade 2	38	25 (65.8%)	<10 – >100
		Chilli grade 3	44	41 (93.2)	<10 – >100
		Cold store	15	12 (80.0%)	<10 – 50
		Chilli powder pepper	43	17 (39.5%)	<10 – >100
Klieber (2000)	Australia	Chilli powder	26	8 (30.8%)	5–10
		Paprika powder	21	5 (23.8%)	5–10
Martins et al. (2001)	Portugal	Cayenne pepper	5	5 (100%)	2–32
Erdoğan (2004)	Turkey (Erzurum)	Red-scaled pepper	44	8 (18.2%)	1.1–97.5
		Powdered red pepper	26	3 (10.7%)	1.8–16.4
		Isot pepper	20	1 (5.0%)	13.8
Food Safety Authority of Ireland (2004)	Europa	*Capsicum* spp. (chilli)	17	1 (5.9%)	<2– 9.7
		Piper spp. (Pepper)	43	43 (100%)	<2–5
Zinedine et al. (2006)	Morocco	Red paprika pepper	14	14 (100%)	2.88–5.40
Food Safety and Inspection Service (2005)	England	Paprika pepper	25	25 (100%)	0.6–3.4
		Cayenne pepper	4	1 (25%)	0.2–6.8
		Chilli powder	28	8 (28.6%)	<0.2–13.9

[a] ND: AFB_1 levels were below minimum detection limit.

Table 2
The AFB_1 levels of powdered red pepper samples

Number of Samples (n)	AFB_1 levels of powdered red pepper samples			
	< 0.025 μg/kg	0.025–5 μg/kg	> 5–40.9 μg/kg	Total x-Sx [a]
100	32	50	18	3.92 ± 0.73

[a] The AFB_1 values, which were determined under minimum detection limit, calculated as "0".

than the results by Ahmad and Ahmed (1995), Fufa and Urga (1996), Klieber (2000), Reddy et al. (2001) and FSIS (2005). In the respect of maximum AFB_1 level results in this study, they were lower than Fufa and Urga (1996), Reddy et al. (2001) results but higher than Wood (1989), Ahmad and Ahmed (1995), Klieber (2000), Martins et al. (2001), FSAI (2004), Zinedine et al. (2006) and FSIS (2005) results. These results showed that AFB_1 occurrence in red pepper in Turkey could relatively be a critical point, regarding the quality of red peppers.

The results of other studies held in Turkey were given in Table 1. It was seen that AFB_1 percentages of red pepper samples, which were investigated in this study, were higher than the results in other studies in Turkey, but lower than the results in Karagöz's study (1999). However, maximum AFB_1 levels, detected in this study, were higher than Erdoğan (2004), Karagöz (1999) and Yıldırım et al. (1997) results. In the perspective of the study, detection of AFB_1 in powdered red pepper has showed that there is not enough precaution on production, transport, harvest, and storage of red pepper. Likewise, Çoksöyler (1999) reported that 1 out 9 pepper samples, which were split and dried on soil and asphalt, had AFB_1 levels of 80 μg/kg, but four samples, which were dried as intact on soil concrete grounds and in string, contained 20–80 μg/kg AFB_1.

In this study, AFB_1 levels of red peppers, which were sold in Istanbul, were found in some of the samples analyzed to be at high-risk levels for human health. It was concluded that, the reason for high levels of AFB_1 is a result of lying red peppers on soil and asphalt for drying, storage of the red peppers under relatively low humidity levels, and insufficient control of transport and shop conditions. The presence of AFB_1 may give rise to high risks to human health because of their carcinogenic, mutagenic and teratogenic effects. As Turkey is one of the leading producer countries of red pepper, more precaution should be taken

on hygiene controls in order to prevent microbiological and chemical hazards. Since red pepper is being used in most of traditional foods in Turkey, further studies should be made on the occurence of AFB_1 in traditional foods.

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