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Original article

INVESTIGATION OF AFLATOXIN B_1 LEVELS IN RED PEPPER AND PRODUCTS CONSUMED IN ANKARA

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Abstract

In this study it was aimed to determine the aflatoxin B_1 (AFB_1) levels in red pepper products and to evaluate whether aflatoxin levels were within the Turkish Food Codex (TFC) values or not. For this purpose, total number of 190 samples consisting 90 red pepper pastes, 50 red pepper sauces and 50 red pepper flakes were collected from supermarkets in Ankara. Determination of AFB_1 levels in red pepper products has been made by immunoaffinity column technique and enzyme-linked immunosorbent assay (ELISA) procedure. Our data revealed that AFB_1 levels were within TFC values except one sample in red pepper products. Furthermore, the examined red pepper products are thought not to have any risk on public health. Although several studies have reported AFB_1 levels in red peppers, this is the first report of AFB_1 levels in pepper sauces and pepper paste consumed widely in Turkey.

Key words: *Aflatoxin B_1, Red pepper products, Immunoaffinity column, ELISA*

Ankara'da Tüketilen Kırmızı Biber ve Ürünlerinde Aflatoksin B_1 Düzeylerinin Araştırılması

Bu çalışmada, kırmızı biber ürünlerinde AFB_1 düzeylerinin saptanması ve bu değerlerin Türk Gıda Kodeksi (TGK) değerlerine uygunluğunun değerlendirilmesi amaçlanmıştır. Bu amaçla Ankara'daki süpermarketlerden 90 kırmızı biber salçası, 50 kırmızı biber sosu ve 50 kırmızı pul biber olmak üzere toplam 190 adet örnek temin edilmiştir. Kırmızı biber ve ürünlerinde AFB_1 miktarlarının saptanmasında immunoaffiniti kolon tekniği ile ELISA yöntemi kullanılmıştır. Sonuçlar değerlendirildiğinde, kırmızı biber örneklerinde, AFB_1 düzeylerinin bir örnek dışında TGK sınır değerleri içinde olduğu saptanmıştır. Ayrıca, analiz edilen kırmızı biber ürünlerinin halk sağlığı açısından risk oluşturmadığı düşünülmektedir. Kırmızı pul biberlerde AFB_1 değerlerini bildiren birçok çalışma olmasına rağmen, bu çalışma, Türkiye'de tüketimi yaygın olan kırmızı biber sosları ve kırmızı biber salçalarında AFB_1 değerini bildiren ilk rapordur.

Anahtar kelimeler: *Aflatoksin B_1, Kırmızı biber ürünleri, Immunoaffiniti kolon, ELISA*

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INTRODUCTION

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus (A. flavus, A. parasiticus* and *A. nomius)* (1,2,3). AFB_1 is known to be carcinogenic and classified by the International Agency for Research on Cancer (IARC) as 1st class carcinogen (4,5,6).

Growth of these fungi on certain foods and feeds may result in aflatoxin production which results in illness or death in humans and animals and thus is an important public health concern (7,8,9). The presence of aflatoxins in dairy products, groundnuts, other edible nuts, dried fruits, figs, sesame and cereals (especially maize) and some spices was investigated in Turkey (8,9,10,11,12,13,14,15,16). In addition, presence of aflatoxins in baby foods and mothers' breast milks was reported (3,17).

In agricultural products, red pepper (*Capsicum annuum*) is cultivated and consumed in a variety of ways in Turkey (18,19). Following China and Mexico, Turkey is the third in production of pepper all over the world (20). In many regions of Turkey, red pepper is consumed as red pepper flakes, pepper paste and pepper sauce (19,20).

Tropical climates with high temperature and humidity are suitable conditions for mycotoxin contamination and spices are mostly produced in countries with tropical climates (21). Spices are exposed to a wide range of microbial contamination as a result of improper production process, extended drying times and poor storage conditions and red pepper flakes is a very sensitive product for aflatoxin formation depending on unsuitable processing conditions (13,22) because they are usually dried on the ground in the open air (21). Red pepper flakes are consumed in Turkey by the majority of people, especially for flavoring, seasoning and imparting aroma or coloring foods (21).

Pepper paste is a traditional food in Turkey and produced generally under the sun in open air in Turkey. Recently pepper paste has been manufactured in a similar way like tomato paste (18). Pepper paste has an important place in Turkish cuisine and is consumed by the majority of people. Furthermore, pepper sauce is also a red pepper product, thus it is also thought to have a risk for AFB_1 and besides red pepper flakes and pepper paste; pepper sauces were also included in the study.

In European countries the maximum level of AFB_1, should not exceed 5µg/kg (ppb) in red pepper and red pepper products. In Turkey, the AFB_1 level should not be greater than 5 ppb in red pepper flakes according to the Turkish Food Codex (TFC) (23). In addition, AFB_1 level should not be greater than 5 ppb for red pepper pastes and sauces according to the TFC. In terms of risky food stuffs, this is the accepted value of TFC for AFB_1. In our study it was aimed to determine the AFB_1 levels in red pepper and products which are widely consumed in Turkey and to evaluate whether aflatoxin levels are within the Turkish Food Codex values or not. There are several reports informing the AFB_1 levels in red pepper flakes from Turkey but this is the first report declaring the AFB_1 levels in red pepper paste and red pepper sauces from Turkey.

EXPERIMENTAL

Samples Collection

Total 190 red pepper products, consisting of 90 red pepper pastes, 50 red pepper sauces and 50 red pepper flakes were investigated for AFB_1 levels. The red pepper products were chosen from different firms; 10 samples were used from one firm and all the samples from the same firm had different serial numbers. All samples were collected from supermarkets in Ankara and originated from Turkey.

Analysis of Samples

Determination of AFB_1 was based on an ELISA using the I'screen AFLA B_1 ELISA kit (TECNA S.r.1., Trieste, Italy) (24). Sample preparations were done according to the instructions of the TECNA kit. The kit was stored at 4°C and all the reagents were brought to room temperature, 2 hours before use.

5 g of the samples were weighed and grinded with 0.5 g NaCl 25 ml 80% methanol and 12.5 ml hexane were added to the samples and centrifuged at 350-400 rpm for 3 minutes. After centrifugation the suspension was filtered through a Whatman-1 filter. 17.2 ml phosphate buffer saline (PBS) (pH:7.2) was added to 2.8 ml of the methanolic lower phase. This emulsion was filtered through glass fiber filter.

Immunoaffinity columns were brought to room temperature before use and the storage solution was eliminated from the columns. 10 ml of the extracts were added (2 ml/minute) to the column and the column was washed with 10 ml PBS. PBS residue was removed from the column with vacuum. Eluate was taken two times with 1 ml of 100% methanol. Methanol was rescued from the column completely. The eluate was evaporated at 40°C and then resuspended with 1.4 ml of 80% methanol. 100 µl of the eluate was diluted with 400 µl of dilution buffer. The final dilution factor is 25.

50 µl of the AFB_1 standards and samples were added to 96 wells microplates. 100 µl enzyme conjugate and 50 µl antibody were added to the wells respectively and incubated for 30 minutes at room temperature. At the end of the incubation the liquid was poured from the wells and washed 4 times with the washing buffer (1:10). 200 µl of the developing solution was added to the wells and incubated at room temperature for 20 minutes. 50 µl stop solution was then added to the wells and measured at 450 nm. AFB_1 concentrations were calculated according to the guidelines of the Tecna kit (24).

RESULTS AND DISCUSSION

The AFB_1 levels were analyzed in the red pepper pastes, pepper sauces and pepper flakes and results was presented in Table 1. Results of the analysis were evaluated according to the guidelines of Turkish Food Codex (TFC).

Our data revealed that AFB_1 mean levels found in all red pepper and products were within the TFC values. In only one sample of 50 red pepper sauces, AFB_1 level exceeded the TFC values while in red pepper flakes and pepper pastes AFB_1 level of all samples did not exceed TFC values (Table1).

Table 1. Levels of AFB_1 in red pepper paste, pepper sauce and pepper flake samples

Type of Sample Analysed	Number of Sample	<1.25 ppb	>1.25-2 ppb	>2-4 ppb	>4-5 ppb	>5 ppb*	Percent
Pepper Pastes	90	69	16	5	-	-	-
Pepper Sauces	50	45	3	1	-	1	2%*
Red pepper flakes	50	11	13	22	4	-	-

*Exceeding TFC values

Red peppers are lying on soil in open air for drying to get red pepper flakes. Additional disposing factors like the inefficient storage conditions and insufficient control of transport and shop conditions affect the occurrence of AFB_1 in red pepper flakes (21).

There are many analytical techniques for the determination of AFB_1 including Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA) in red peppers. However, ELISA was used because of the advantage in terms of simplicity, rapidity, reliability, sensitivity and is cost-effective (2,13,25,26,27). Therefore, ELISA was used for analysis of aflatoxin in present study, coupled with immunoaffinity column (IAC). The employed column contains antibodies specific to aflatoxins, highly efficient for the purification of aflatoxins (25).

In Turkey there are several reports indicating the importance of the presence of AFB_1 in red pepper flakes. Many researchers reported the levels of AFB_1 in red pepper powder or chilli powder and red pepper flakes. Among these, there are some data reporting higher levels than those reported in the present study.

Omurtag et al (28) determined 65% total aflatoxin contamination rate in 26 dried red pepper samples from various cities of Turkey and of these samples, in 43.2% total aflatoxin level higher than 5 ppb was reported using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) methods. Taydaş and Aşkın (29) reported AFB_1 levels as 0-264 ppb in 83 red pepper samples from a total of 127 samples by TLC and fluorescence spectrophotometer. Bircan (30) identified AFB_1 range levels as 0.5-116.4, 1.6-80.4 and 0.3-1.2 µg/kg, respectively in paprika, chilli powder and ground black pepper samples using the IAC and HPLC methods. Ağaoğlu et al (26) determined the AFB_1 levels in red pepper flakes between 1.10-44.0 µg/kg (ppb) by TLC and reported that among the studied red pepper flakes, 57.5% had higher AFB_1 levels than the acceptable levels of the Turkish Food Codex and the highest AFB_1 contamination level was reported to be 44.0 ppb in red pepper flakes. Erdoğan (31) studied 44 red-scaled peppers and 26 red powder peppers, as well as 20 isot samples from various spice retailers in Erzurum, employing TLC. This study reports the presence of aflatoxin (B+G) in 8 red peppers (18.2%), 3 red powder peppers (10.7%), and in 1 isot sample (5%), respectively. Heperkan and Ermiş (32) examined a total of 36 samples of red pepper grown in 4 different regions of Turkey using HPLC method and detected AFB_1 only in 5 samples at levels between 10.5 and 31.2 ppb. Set and Erkmen (33) studied AFB_1 levels in ground red peppers by HPLC and determined 19 out of 71 unpackaged samples and 1 out of the 37 packaged samples exceeded limit value (5ppb). Ardıç et al (21) studied 75 samples of deep-red ground pepper (isot) marketed in Şanlıurfa (Turkey) which were purchased from bazaars and herbal shops, using ELISA coupled with immunoaffinity column and determined that 72 of the 75 ground deep-red pepper samples (96%) contained AFB_1 in the range of 0.11–24.7 µg/kg and 11 (14.7%) samples were above the regulatory limits used in the European Union and in Turkey. Aydın et al (7) reported that 18% of the powdered red pepper samples had unacceptable AFB_1 levels according to Turkish Food Codex values by ELISA. In the same study the highest AFB_1 level that they determined was reported to be 40.9 µg/kg. Kanbur et al (27) determined the AFB_1 levels in red pepper flakes by enzyme immunoassay, between 1.48-70.05 ppb. In 3 samples that had higher AFB_1 levels than the TFC maximum limits, the AFB_1 levels were 5.39 ppb, 5.67 ppb and 70.05 ppb, respectively. Kurşun and Mutlu (16) determined the total aflatoxin range levels by ELISA, in red pepper to be as 3.55-9.55 µg/kg. Çolak et al (13) studied AFB_1 by HPLC and ELISA. They determined that 36 out of 84 spice samples (42.9%) were found to be contaminated with aflatoxins in the range of 0.3-46.8 µg/kg. According to these results, 9 red-scaled and 3 red pepper samples exceeded the maximum limits of AFB_1 (5 µg/kg) and total aflatoxin (10 µg/kg) set in the TFC.

These findings are higher than the AFB_1 levels determined in the present study. The reason for this is thought to be because of the sample distribution. In our study all the red pepper flakes samples were commercially packaged samples from five different firms. Therefore, packaging is also a good process to supply attractive and healthy products in

hygienic conditions (16,31). Moreover, it might be thought that hygienic conditions due to package of food products may be important to reduce or prevent contaminations of aflatoxins and others.

In our study, low levels of AFB_1 in red pepper and products are thought to be because of packaging that provides hygienic conditions and prevents the samples from external influences. These results also support the importance of storage, transport and shopping conditions for aflatoxin contamination in red pepper products. Set and Erkmen's study results with unpackaged and packaged samples supports these items (33).

The level of AFB_1 in red pepper products was investigated in several countries. Romagnoli et al (34) reported that only 7 of 103 spices that collected from Italy were aflatoxin positive by HPLC. Iqbal et al (35) detected total aflatoxins in 26 (33%) of whole chillies by HPLC and reported the concentration range from 0.00–81.5 μg/kg in Pakistan. Santos et al (36) studied 64 paprika samples and reported that 59% were aflatoxin positive, whereas in the 35 chilli samples, the contamination was 40% for aflatoxin and determined that none of the samples had aflatoxin levels higher than the legally allowable limits in Spain by HPLC. Shundo et al (37) studied 70 paprika samples in Brazil and aflatoxins were found in 82.9% of the samples. They also reported that AFB_1 was detected in 61.4% at levels ranging from 0.5 to 7.3 μg/kg with mean concentration of 3.4 μg/kg using an IAC and HPLC. Martins et al (38) studied 79 spice samples by using IAC and HPLC in Portugal and determined that 43% of the samples were positive for AFB_1. Zinedine et al (39) determined AFB_1 mean levels as 2.88 μg/kg and reported that the higher level of contamination was found in red paprika (9.68 μg/kg) by using IAC and HPLC. Riordan and Wilkinson (40) studied the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market by HPLC and IAC and out of 30 chilli powder samples 10 were reported to be positive in case of AFB_1 presence and the mean of AFB_1 levels were reported to be 9.05 ± 8.67 ppb. In Hungary, Fazekas et al (41) reported that 18 of the 70 ground red pepper samples contained AFB_1 (25.7%) and 7 samples (10%) contained AFB_1 in excess of the 5 μg/kg maximum level in concentrations ranging from 6.1 to 15.7 μg/kg by using HPLC. Cho et al (25) studied spices by HPLC in Korea. They investigated 41 red pepper powder samples and 15 red pepper paste samples. Seven red pepper powder samples out of 41 red pepper powder samples and 2 red pepper paste samples out of 15 red pepper paste samples were reported to be contaminated with AFB_1. The AFB_1 level range was determined to be between 0.08-4.45 ppb for red pepper powders and 0.21-0.55 ppb for red pepper pastes. Shamsuddin et al (42) examined 176 samples by TLC for AFB_1 contamination and determined that 66% of the red chilli studied were contaminated with AFB_1. Reddy et al (43) collected chilli powders from different supermarkets in Hyderabad and analyzed the samples for AFB_1 content by an indirect competitive ELISA. Of the 182 chilli samples tested, 59% of the samples were contaminated with AFB_1 and 18% contained the toxin at non-permissible levels. The highest AFB_1 concentration of 969 μg/kg was found in 1 sample.

In foreign countries different levels than our results, in aflatoxin were detected in red pepper and products. The differences in the results are thought to be because of climatic and regional factors besides the red pepper type and manufactural processing.

There are several studies in Turkey, investigating aflatoxin presence, reporting a risk for public health (2,3,8,10,12,13,14,44). AFB_1 is reported to be a potential hazard for animal and human health and is the most known potent carcinogen (13,17,45) According to the current study, the examined red pepper products are within TFC values except for one pepper sauce sample. However, the average daily intake of AFB_1 can increase through consumption in many foods contaminated with AFB_1, thus it is thought to have a risk on public health.

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