

Investigation of Aflatoxin B1 Levels in Red-scaled Pepper by ELISA

Kırmızı Pul Biberlerde Aflatoksin B1 Miktarının ELISA Yöntemi ile Araştırılması

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ABSTRACT

Objective: Mycotoxins have carcinogenic, mutagenic and teratogenic effects on most animals and humans. Among all mycotoxins, aflatoxin B1 (AFB1) has the most toxic effects.

Methods: In this study, the aflatoxin B1 levels in red-scaled pepper samples have been determined by ELISA method.

Results: The results revealed that 69 out of 82 samples (84.14%) were found to be contaminated with aflatoxin B1 in the range of 5.1-20.94 $\mu\text{g kg}^{-1}$ (ppb).

Conclusion: Aflatoxins are dangerous contaminants of red pepper and have to be considered as a major concern for public health.

Key Words: Aflatoxin B1, red scaled pepper, ELISA, mycotoxin, public health

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ÖZET

Amaç: Mikotoksinlerin, hayvan ve insanlarda karsinojenik, mutajenik ve teratojenik etkileri bulunmaktadır. Mikotoksinler arasında en toksik etkiye sahip olan aflatoksin B1 (AFB1)'dir

Yöntem: Bu çalışmada, kırmızı pul biber örneklerinde aflatoksin B1 miktarları ELISA yöntemi ile araştırılmıştır.

Bulgular: Sonuçlar, incelenen 82 adet örneğin 69 tanesinin (%84,14), 5.1-20.94 $\mu\text{g kg}^{-1}$ (ppb) değer aralığında aflatoksin B1 kontamine olduğu bulunmuştur.

Sonuç: Aflatoksinler, kırmızı biberlerde kontamine olan tehlikeli bir metabolit olup halk sağlığı açısından çok önemli bir kaygı oluşturmaktadır.

Anahtar Sözcükler: Aflatoksin B1, kırmızı pul biber, ELISA, mikotoksin, halk sağlığı

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INTRODUCTION

Mycotoxins are toxic metabolites produced by some common species such as *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins have carcinogenic, mutagenic and teratogenic effects in most animals and humans (1). Hepatocellular carcinoma (HCC) accounts for 5.5% of all cancer cases worldwide and dietary aflatoxin exposure has been reported to be the two major etiological risk factors for the development of HCC (2).

The aflatoxins were isolated and characterised after the death of more than 100.000 turkey poult (turkey X disease) in England during 1960 (3). Aflatoxins can be produced both pre-harvest and during storage of cereal, rice, wheat, red-scaled pepper and nuts (4). Eighteen different types of aflatoxins were identified. Among all aflatoxins, aflatoxin B1 (AFB1) has the most toxic effect, followed by the aflatoxins G1, B2 and G2, respectively (5). According to the International Agency for Research and Cancer (IARC), AFB1 is the most carcinogenic mycotoxin (6).

Valuable export material is under the risk of being contaminated by aflatoxins during production procedures. Especially being dried on the ground in the open air in poor hygienic conditions promotes the production of mycotoxins (7).

The probable amount of aflatoxins in red-scaled pepper is important for public health and economy. The allowed maximum legal limits of total aflatoxin (AFT) and AFB1 for ground red pepper are the same in Turkey (8) and the European Union (9) and reported as 10 ppb for AFT and 5 ppb for AFB1. Enzyme-linked immunosorbent assay (ELISA), is a fast screening method with good specificity, sensitivity and simplicity for the determination of aflatoxins (10).

The aim of this study was to determine the levels of AFB1 in red-scaled pepper samples by ELISA.

MATERIALS AND METHODS

Samples

A total of 82 red-scaled pepper samples, unpacked (n=78) and packed (n=4), obtained randomly from supermarkets and bazaars in Ankara. All samples had been collected into sterile jars from different sales points. All of the samples were analysed by an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices VersaMax microplate reader) and AFB1 test kit (Immunoscreen AFLA kit B1, Tecna Code MA 314) were also used to run ELISA reader.

Preparation of samples

A hundred ml of 80% methanol were added to 20 g red-scaled pepper sample and mixed by vortex. Samples were centrifuged at 5000 rpm for 5 min. A hundred µl of the supernatant were mixed with 400 µl of the dilution buffer. A hundred µl of this mixture was added to 100 µl of a 16% solution of methanol in dilution buffer. Samples were centrifuged at 10.000 rpm for 5 min and, at this point, diluted supernatant was ready for the assay.

Assay Procedure

Fifty µl of each standard/sample were added into the standard wells. Using the multichannel micropipette, 100 µl of enzyme conjugate was added into each well. Then, 50 µl of antibody was also added in each well except the blank wells. The plates were shaken gently with a rotatory motion for a few seconds. The plate was incubated for 30 min at room temperature. At the end of the incubation period, washing sequences were repeated 4 times and 200 µl of developing solution was added to each well and incubated for 20 min at room temperature. Finally, 50 µl of stop solution was added to each well and mixed thoroughly with rotatory motion for a few seconds. Absorbance of the samples was measured at 450 nm.

Calculation

The mean absorbance values of samples and standards were calculated according to this formula:

$$\frac{\text{absorbance standard (or sample)} / \text{absorbance standard 0 (B0)}}{100} \times 100 = B/B0 (\%)$$

The mean absorbance value of the blank was subtracted from the mean absorbance value of maximum binding (B0) and all the standards and samples. The findings were divided by the absorbance value of maximum binding and were multiplied by 100. Maximum binding was made equal to 100% and the absorbance values were quoted in percentages. The mean values of the absorbances for the standards and the samples were evaluated according to excel spreadsheets downloaded from www.tecnalab.com.

RESULTS

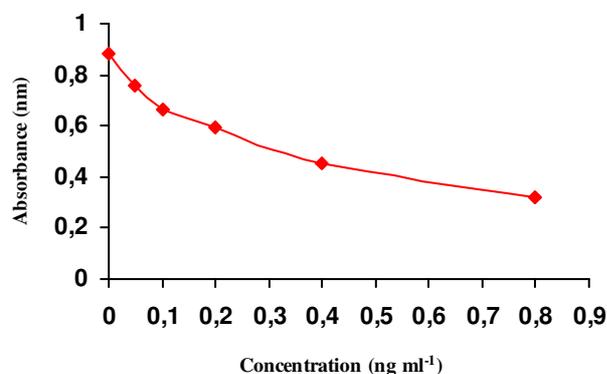
According to the Turkish Food Codex, 69 of 82 red-scaled peppers samples (84.14%) were found to be contaminated with AFB1, whereas 13 of 82 red-scaled peppers samples (15.86%) were found not to be contaminated with AFB1 (Table 1). Four of the packed samples were also found to be contaminated with AFB1 ranged between 6.14-17.86 µg kg⁻¹. The levels of AFB1 ranged between 5.1-20.94 µg kg⁻¹ in the positive samples. The maximum AFB1 levels exceeding the legal limit in six of the samples are shown in Table 2 (*Turkish Food Codex 2008*). Also, the AFB1 levels ranged between 1.62-4.94 µg kg⁻¹ in the negative samples. AFB1 contamination for each samples were calculated according to standard curve (Figure 1).

Table 1. The number and percentage of unpacked and packed samples contaminated with AFB1.

Type of sample	Number of samples with AFB1 (%)	
	<5 ppb	>5 ppb
Unpacked (n = 78)	13 (16.66%)	65 (83.34%)
Packed (n = 4)	-	4 (100%)
Total (n = 82)	13 (15.86%)	69 (84.14%)

Table 2. The maximum AFB1 levels exceeding legal limit (>5 µg kg⁻¹) in the samples (n=6).

Sample No	Level ppb (µg kg ⁻¹)
18	20.22
19	20.48
35	20.22
43	20.28
62	20.94
73	20.38



Standard	S1	S2	S3	S4	S5	S6
Concentration (ng ml ⁻¹)	0	0,05	0,1	0,2	0,4	0,8
Absorbance (nm)	0,885	0,754	0,663	0,597	0,450	0,320

Figure 1: Calibration curve of an Immunoscreen AFLA kit B1 and reference values.

DISCUSSION

Aflatoxins have been reported to be a significant public health concern, and have been associated with human aflatoxicosis, neural tube defects, and many types of primary cancers (11).

High AFB1 levels had been determined in 57.5% of the tested red-scaled peppers (12). It had been reported that 42.9% of the peppers were contaminated with AFB1 with a range of 0.3-46.8 µg kg⁻¹ (1).

AFB1 had been found to be at 18.2% in red-scaled pepper ranging between 1.1-97.5 $\mu\text{g kg}^{-1}$ (13). Also, the red pepper samples had been reported to be contaminated with AFB1 levels ranging from 3.55-9.55 $\mu\text{g kg}^{-1}$ by ELISA (14).

It has been reported that 43% of the red ground peppers were contaminated with AFB1 in the range of 1–20 $\mu\text{g kg}^{-1}$ in Portugal (7). Also 18% of the ground red pepper samples had been found to be contain AFB1 above 5 $\mu\text{g kg}^{-1}$ (15). It had been shown that 13.3% of ground red pepper samples were contaminated with AFB1 in concentrations of 250–525 $\mu\text{g kg}^{-1}$ (16). The range of contamination of pepper with AFB1 has been reported to be between 0.65-2.1 $\mu\text{g kg}^{-1}$ in Malaysia (17).

Our study revealed that 84.14% of red-scaled peppers were found to be contaminated with AFB1 ranging between 5.1-20.94 $\mu\text{g kg}^{-1}$. These levels are above the allowed maximum legal limits of AFB1 both for Turkey and the European Union.

CONCLUSION

Aflatoxins are dangerous contaminants of red pepper and have to be considered as a major concern for public health. So, starting from the production, process methods including harvesting, drying, packaging, transportation and also storage have to be monitored by a reliable method such as ELISA.

Conflict of interest

No conflict of interest was declared by the authors

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