Aflatoxin B1 Quantitative Analysis in Red Peppers for Consumption in Kayseri Using Immunoassay Enzyme (EIA)

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Abstract. Aflatoxins (AF) are extremely toxic and carcinogenic compounds and are metabolites of mold fungi Aspergillus flavus and Aspergillus parasiticus. Food and feed can be contaminated with these molds during harvest, storage, processing and marketing. In this study, aflatoxin B1 (AFB1) in 50 red pepper samples sold in Kayseri was analyzed by EIA method. The presence of AFB1 was found in all of the samples. AFB1 values were determined in the range of 1.48-70.05 ppb. It was determined that three of the red pepper samples (6.0%) had AFB1 higher than the level allowed in spices (5 ppb) according to the Turkish Food Codex Regulation.

Keywords: Aflatoxin B1, Analysis, Enzyme Immuno Assay, Red Pepper, Kayseri, Turkey.

A. INTRODUCTION

AFs are substances produced by various fungi, which can cause acute, subacute and chronic poisoning when eaten by humans and animals. AF is the substance with the highest potential to cause liver cancer in humans and is considered as group I carcinogens by the World Cancer Research Center (IARC) (13,15). Apart from liver cancer, AFs also have many important toxic effects such as suppressing the immune system and causing deterioration in protein metabolism (19).

Fungi that produce AF can be found in the flora of agricultural products and various foodstuffs, or they can infect these products in different ways; when suitable conditions are created for their development, it causes the formation of AF along with deterioration in agricultural products and foodstuffs (18). Red pepper is one of the products sensitive to AF formation due to the conditions it faces during production, harvest, drying and further processing (7,11,18).

Pepper is a cultural plant that is grown in open and greenhouse in various countries of the world and is important for the consumer, producer and processing industry.

The largest country in world pepper production is China, followed by Mexico and Turkey, respectively. Pepper is an important product for the country's economy, as Turkey alone has a share of 8% in world pepper production and 3% in world processed pepper trade. (9).

The occurrence of AF in red pepper in our country has attracted attention with the return of exported peppers from European countries for the last 10-12 years (11). According to the Turkish Food Codex Regulation, the amount of AFB1 allowed in spices is 5 ppb. (3). The level of AFB1 allowed in spices in the European Union is 5 ppb (4).

There are various studies conducted in the world and in Turkey to determine the AFB1 content of red peppers for consumption. In a study conducted by El-Kady et al. (10) in Egypt, they determined AFB1 levels of 8 -35 ppb in 16 (13.3%) of 120 different spice samples. Fufa and Urga
(12) reported that 8 (13.33%) of 60 red pepper samples collected from markets in Ethiopia had AFB1 in the range of 100-500 ppb. In the study conducted by Reddy et al. (17), it was determined that 59% of 182 red pepper samples collected from the markets in India were contaminated with AFB1, and the AFB1 level in 18% of them was above the accepted limits (30 ppb).

Taydaş and Aşkın (18) determined that 83 (65.3%) of 127 red pepper samples had AFB1 between 1.25-28.50 ppb. In a market survey conducted by the Ministry of Agriculture, it was stated that 46.7% of powdered and red chili peppers contain more than 5.0 ppb of AFB1 and that peppers are the most contaminated product in terms of AFB1 (2). Ready and Çoksözler (15) determined AFB1 in the range of 0.45-80.25 ppb in 46 (32.6%) of 141 red pepper samples. In the research conducted by Ağaoğlu (1), 40 red pepper flakes samples sold in the open in the province of Van were examined in terms of AFB1; AFB1 was detected in all of the samples as a result of the study; 1.10 -5.00 ppb in 42.5% (17 samples) of samples, 5.01-10.00 ppb in 40% (16 samples), 10.01-20.00 ppb in 12.5% (5 samples) and 20.01- in 5% (2 samples) AFB1 was detected between 44.00 ppb. Erdogan (11), in his AFB1 screening on 90 pepper samples, found that 12 of the samples (13.3%) contained AFB1 at the level of 1.1-97.5. Aydın et al. (5) analyzed 50 red pepper powder samples from various companies offered for sale at different points in Istanbul and found that 16% (8 samples) were below 1.0 ppb and 72% (36 samples) 1- They detected AFB1 at 5 ppb and over 5 ppb in 12% (6 samples).

EIA is widely used in AF analyzes in spices because it is easy to apply, relatively inexpensive, and has high sensitivity (6,14,17,18). With this research, it was aimed to determine the AFB1 contamination levels of red pepper samples offered for consumption in Kayseri and to evaluate the results according to the limits specified in the Turkish Food Codex Regulation.

B. METHOD

Within the scope of the research, 50 red pepper samples sent for analysis from various institutions in Kayseri to the Department of Pharmacology and Toxicology in 2004 were analyzed in terms of EIA and AFB1. Each sample was analyzed 3 times and these were averaged. Results are given in ppb. The AFB1 values found were evaluated according to the Turkish Food Codex limit (5 ppb). Calculations were made with Excel XP program.

AFB1 standards of 0.0, 0.5, 1.5, 4.5, 13.5 and 40.5 ppb were prepared in methanol and the analyzes were determined by EIA using the r-Biofarm kit. The results were calculated as ppb in kineticCalc.V2.16 program. Excel XP program was used in the calculations of the recovery value, correlation coefficient and calibration equation of the method.

In analysis; 2 g of red pepper sample was taken into a beaker and mixed with 10 ml of 70% methanol at room temperature for 10 minutes. The mixture was filtered through a disposable C18 column. AFB1 analyzes of red peppers were performed by EIA using the r-Bipharm kit. The results were calculated as ppb in kineticCalc.V2.16 program.

C. RESULT AND DISCUSSION

It was determined that the recovery of the method was 85% and the correlation coefficient was 0.9998. The calibration line equation of the method (y = mx + n) is y = 1.1486x + 0.0205 (y = absorbance, m = slope, n= cut, x = concentration). As a result of the analysis of the samples, the presence of AFB1 was found in all of the samples. AFB1 values in red peppers were determined in the range of 1.48-70.05 ppb (Table 1). In 3 (6.0%) of the pepper samples, AFB1 was detected over 5 ppb, including 5.39, 5.67 and 70.05 ppb.
In the acceptability of the methods used in AF studies, recovery of 70% and above (18); In analytical studies, it is desired that the correlation coefficient (r) be very close to +1 and -1 (13). In this study, red pepper AF analyzes were performed with EIA and the recovery of the method was 85% and the correlation coefficient was 0.9998. The calculated recovery rate and correlation coefficient are within the accepted ranges.

In the Southeast Anatolia Region, which is the region where red pepper production is the most in our country, some producers dry the red peppers on the soil and then sell them to the factories (15,18). It is thought that red pepper produced under these conditions will be a very open product to soil-born fungal contamination and AF formation. In the production of red pepper, the inner cavity of the dried red peppers without opening creates a suitable environment for fungal colonization and therefore for the formation of AF (7,8,18). In this study, the determination of AFB1 at the level of 1.48-70.05 ppb in red peppers and the detection of AFB1 over 5 ppb in 6% of the samples can be explained by the fact that some producers in the production of red peppers dried the peppers on the ground or without opening.

The rate of AF is higher in red peppers produced from peppers grown especially in humid, rainy and temperate climates (19). El-Kady et al (10), Fufa & Urga (12), Reddy et al. (17) found that the high levels of AF in red pepper samples were determined by unsuitable production conditions, humid-temperate climatic conditions in the regions where peppers are grown, and peppercorns. It is stated that it is closely related to production techniques. 80% of the red pepper production in our country is made in the provinces of Kahraman-Maras and Gaziantep (15). Although the southern parts of these provinces are under the influence of the Mediterranean climate, in general, summers are hot and dry, and winters are cold and rainy. Kayseri region has continental climate conditions. The determination of AFB1 in all samples and the presence of samples containing more than 5 ppb of AFB1 in our study may be related to the use of traditional production methods and post-production storage conditions in these regions rather than the climatic conditions of the region.

D. CONCLUSION

According to the Food Codex Regulation, a maximum of 5 ppb AFB1 is allowed in spices (3). In studies conducted in Turkey, 1.25-28.50 ppb in 65.3% of Taydaş and Aşkın (18) samples, Ministry of Agriculture (2) in 46.7%. It was determined that AFB1 was over 5.0 ppb, 0.45-80.25 in 46.0%, Ağaoğlu (1) 1.10-44.00 ppb, Aydin et al. (5) over 5.0 ppb in 12.0% of Ready and Çoksözler (15). In these studies, it is remarkable that there are significant differences between the lowest and highest AFB1 values in the samples and the percentage of AFB1 over 5.00 ppb. Stated to be due to packaging. In our study, it is thought that the determination of AFB1 in the range of 1.48-70.05 ppb in all of the samples may be due to unsuitable production and storage conditions, as well as the packaging of the sample from a region containing high amounts of AF. In our study, the percentage of AFB1 over 5.00 ppb in pepper samples was quite low (6.0%) when compared to other studies.

In case AFs are taken in low amounts and for a long time, in addition to increasing the risk of liver cancer, they can also cause immunosuppression and deterioration in protein, vitamin A, vitamin D, iron, selenium and zinc metabolism, and may increase the incidence of malaria (19). As a result of this research, it can be said that consuming red pepper samples containing more than 5 ppb of AFB1 may cause significant health problems. Red pepper is a widely consumed product with export value in Turkey. For this reason, peppers containing high levels of AFB1 may cause significant problems and economic loss in terms of both human health and exports. As a result, the
contamination of red peppers produced in our country with AF still continues to be an important health and export problem. These problems can be solved with the care to be shown during the production, storage and sale of red pepper, by analyzing the red peppers in terms of AF before they are offered for consumption and by market controls to be made after production.

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