PAPER

THE CONTAMINATION RATE OF AFLATOXINS IN GROUND RED PEPPERS, DRIED FIGS, WALNUTS WITHOUT SHELL AND SEEDLESS BLACK RAISINS COMMERCIALIZED IN SAKARYA CITY CENTER, TURKEY

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ABSTRACT

The investigation of this study is concerned with the occurrence of aflatoxin total (AFT) and aflatoxin B1 (AFB1) in 120 specimens randomly bought from retail stores, bazaars, supermarkets and regional stores in Sakarya City center. Immune affinity (IAC) clean-up with high-performance liquid chromatography (HPLC) and fluorescence detection (FID) methods were used to investigate the specimens to determine the incidence of aflatoxin (B1, B2, G1, G2) contamination. The findings indicate that the percentages of ground red peppers, dried figs, walnuts without shell and seedless black raisins contaminated with AFT are about 72%, 51%, 64% and 64%, respectively. One ground red peppers (18.68-10.49 μ g/kg) and one specimen of walnuts without shell (10.26-5.06 μ g/kg) exhibit the maximum contamination levels of AFT and AFB1, respectively. Of all the contaminated specimens, two ground red peppers and one dried fig specimens (2.5%) exceed the recommended AFB1 (5 μ g/kg) limit defined by the Turkish Food Codex (TFC) regulations. This study presents the details of the first inspection regarding the presence of aflatoxins in seedless black raisin specimens in Sakarya City center of Turkey.

Keywords: aflatoxin, HPLC-FLD, pepper, fig, walnut, raisins

1. INTRODUCTION

As secondary metabolites, mycotoxins are generated by micro fungi which result in vertebrates contracting illnesses ((LUTTFULLAH and HUSSAIN, 2011). Aflatoxins (AFs) from mycotoxin family are immensely deleterious secondary metabolic compounds of Aspergillus flavus, A. parasiticus and A. nomius (SET and ERKMEN, 2010). They can cause cancer, mutations and physiological abnormalities in animals and humans (ERKMEN and BOZOGLU, 2008). AFs are impossible to avoid because they are found in nature as contaminants (IARC 1993). External circumstances such as climate, moisture and the span of precipitation during tilling and crop yield seasons affect the contamination (DINI et al., 2013). Such products as cereals, oil seeds, nuts, beans and spices are affected the most (REDDY *et al.*, 2011). As a toxic chemical, AFB1 is one of the most potent substance which causes cancer and is categorized group I by International Agency for Research on Cancer (IARC 1993). The scale of toxicity, AFB1>AFG1>AFB2>AFG2, indicates that the fatal furan moiety of AFB1 is the significant point to detect the stage of bioactivity of this group of mycotoxins (COLAK et al., 2006). Numerous researches demonstrate that gastrointestinal diseases, hepatic neoplasms and hepatocellular carcinoma observed in humans in Africa, Philippines and China are related to aflatoxins (LUTTFULLAH and HUSSAIN, 2011).

Additionally, it has been manifested that feeding on products polluted by aflatoxin has led to some aflatoxicosis-related epidemics (REDDY and RAGHAVENDER, 2007). Aflatoxicosis-related epidemics were observed to affect a large geographical area and result in the demise of 123 people in Kenya (CDC, 2004).

It is of great importance to safety not only the well-being of people but also the interests of producers by applying an array of action to decrease the aflatoxin contamination to the minimum. If this is not achieved, the situation can have debilitating repercussions for producers in terms of their economic perpetuity and can also debar people from a substantial food source (DINI *et al.*, 2013). Some studies and investigative research have been conducted in many countries to acquire a prevalent paradigm as regards foods contaminated with aflatoxin (REDDY *et al.*, 2011). Owing to the fact that AFs are toxic and observed quite often, many countries, including Turkey, have outlined protocols and tolerance margins for aflatoxins. The Turkish Food Codex (TFC) states that the maximum levels for AFB1 and AFT in dried fruits are 8 and 10 μ g/kg, in nuts or peppers 5 and 10 μ g/kg, respectively (TFC, 2011).

Owing to the considerable risks of health complications caused by aflatoxins in foods, it is of utter importance to collect data regarding the prevalence of these toxic substances in foods in Sakarya (Turkey). Despite a lot of studies on aflatoxin rates in a variety of food which are present in the diet of people in Turkey, the research on aflatoxins in ground red peppers, figs and walnuts in Sakarya is scarce. Therefore, the aim of this study is to specify the AFT (AFB1, AFB2, AFG1, and AFG2) and AFB1 contamination rates in ground red peppers, walnuts without shell, dried figs and seedless black raisins, to show how contamination is a risk to the public health and to compare the findings to the highest aflatoxin tolerance margins defined by the TFC. This is also first survey to determine the incidence of AFs contamination in seedless black raisins in Sakarya city.

2. MATERIALS AND METHODS

2.1. Specimen preparation

120 food specimens were purchased in 2014 and 2015 from different retail shops, bazaars, supermarkets and local markets in Sakarya City center, Turkey. While a few of the

specimens were foreign origins, the majority of the specimens were from Turkey. They have been produced traditionally and locally consumed in generally. Most of the specimens sold in bulk were purchased unpackaged except for a few. The selected commodity groups are 25 ground red peppers and dried fruits including 45 dried figs, 25 walnuts without shell, 25 seedless black raisins (each about 500g). A subsample divider was used to divide the specimens. A 200 g subsample was ground and placed in plastic bags and kept at -20°C until they were analyzed and put away to avoid sunlight. Two specimens were selected and two separate analyses were carried out for each specimen (SET and ERKMEN, 2010). Mean results of the four analyses were presented. All the testing and fluxing agents were of LC grade delivered from Merck (Darmstadt, Germany) and Sigma (St. Louis, USA). The aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2 (AFB1, AFB2, AFG1, AFG2) mix standard were obtained from Supelco (Sincer, Turkey). Standard stock solutions (2600 ng/mL) of mixed aflatoxin were produced using methanol, covered with aluminium foil so that aflatoxins would steadily dissolve under ultraviolet light and be kept for up to 3 months. The stock solutions were thinned to the rates of 0.3 and 1.0 μ g/mL (standard solutions) using methanol and kept at -20°C (SET and ERKMEN, 2010).

2.2. Aflatoxin analyses in specimens by HPLC

This study was carried out making a few adjustments using HPLC method as described by AOAC (2000) and STROKA *et al.* (2000). Briefly, 5 g of NaCl was put in 50 g of walnuts without shell and afterwards mixed with 200 mL of methanol/water (80:20) and 200 mL of cyclohexane for 3 min. following the parting of the two phases, cyclohexane was done away with. For dried figs, ground red peppers and raisins, specimens were removed only with 200 mL of methanol / water (80:20). Extracts were filtrated using a Whatman filter paper No. 4 with a pore size of 30 um (LUTTFULLAH and HUSSAIN, 2011). 10 mL filtrate was thinned using 40 mL Phosphate buffer saline (PBS) from Sigma (St. Louis, MO, USA). 10 mL of filtrate was sent through the immune-affinity column (AflaPrep, R-Biopharm Rhone, Scotland) at a speed of 2-3 mL/min. The column was cleaned using 20 mL distilled water. Finally, bounded aflatoxins were eluted slowly using 1 mL methanol and air was pushed through the column to collect the last drops of eluate and finally thinned using 1 mL water (ÖZKAN *et al.*, 2015). The extract was moved to a 1.8 mL vial for the injection (SET and ERKMEN, 2010).

The mobile phase was water-methanol-acetonitrile (5:2:3, v/v/v). The final concentration of the mobile phase was set to be 88.23 mg/L and 120 mg/L by nitric acid (LC grade, Sigma-Aldrich, Germany) and potassium bromide (LC grade, Merck, Germany) respectively. The mobile phase was filtered through a disposable filter unit (0.45 μ m). The presence of aflatoxins was observed by HPLC (Shimadzu, Tokyo, Japan) using a post-column derivatization electrochemically generated bromine (Kobra cell) and a fluorescence detector (RF 20A) at 362 nm (excitation) and 450 nm (emission) (SET and ERKMEN, 2010). The Intersil ODS-3 (25 cm - 4.6 mm ID, 5 μ m, Tokyo, Japan) column was injected to HPLC automatically. The peaks were then compared with the actual specimen peaks obtained with that of aflatoxin standards.

The recovery studies were carried out by spiking to uncontaminated spicemens with two concentration levels of each toxin (AFT and AFB1) at least 1 hour prior to analysis. The recovery rate of aflatoxins was determined at a rate of 5.2 μ g/kg and 2.6 μ g/kg in all specimens in triplicate. The fortified spicemens were extracted and analyzed. The limits of detection (LOD) and the limits of quantification (LOQ) were defined according to signal to noise ratio; S/N=3/1 and S/N=10/1, respectively.

3. RESULTS

This study examined AFT and AFB1 contamination in 25 ground red peppers, 25 walnuts without shell, 25 seedless black raisins and 40 dried fig specimens. The findings were assessed in line with the legal limits for AFT and AFB1 specified by the Turkish Food Codex (TFC 2011).

The following recovery rates were obtained as $\mu g/kg$ in ground red peppers and dried figs respectively: AFB1, 99.8%-106.3%; AFB2, 100.6%-100.1%; AFG1, 99.0%-98.2% and AFG2, 92.3%-102.2%. The limits of detection were as follows: AFB1, 0.05 $\mu g/kg$; AFB2, 0.02 $\mu g/kg$; AFG1, 0.04 $\mu g/kg$ and AFG2, 0.05 $\mu g/kg$. The following recovery rates were calculated as $\mu g/kg$ in walnuts without shell and seedless black raisins respectively: AFB1, 77.4%-84.0%; AFB2, 85.1%-107.0%; AFG1, 99.0-77.7% and AFG2, 75.9%-108.2% (Table 1).

Table 1. Recoveries of aflatoxins in the fortified specimens (%).

Commodities	Mean recovery ^a ± RSD ^b (%)						
	AFB1	AFG1	AFB2	AFG2			
Ground red peppers	99.8±1.5	99.0±4.2	100.6±2.1	92.3±3.4			
Walnuts without shell	77.4±3.7	99.0±4.5	85.1±2.0	75.9±2.7			
Seedless black raisins	84.0±1.8	77.7±5.3	107±3.7	108.2±1.8			
Dried figs	106.3±2.1	98.2±5.3	100.1±3.4	102.2±4.1			

^aNumber of replicates: N= 3; ^bRelative standard deviation.

Table 2 presents the summary of the results of 120 specimens for AFT. The results clearly show that 18 ground red peppers, 23 dried fig specimens, 16 walnuts without shell and 16 seedless black raisins are contaminated with aflatoxins. Of all the specimens, ground red peppers have the highest contamination rates. The mean values of ground red peppers, walnuts without shell, dried figs and seedless black raisins are as 2.30 μ g/kg, 1.68 μ g/kg, 0.40 μ g/kg, 1.78 μ g/kg, respectively. AFT was observed in 73 specimens (68%) and 69 specimens had contamination below rates varying between 0.02 and 3.47 μ g/kg; while 3 specimens had contamination varying between 5.30 and 18.68 μ g/kg, exceeding the recommended limit for AFT.

Table 2. Total aflatoxins in the specimens.

Contaminated specimens with AFT (μ g/kg)							
Commodities	Number of specimens analyzed /positive	Frequency (%)	Below limit (<10 µg/kg)	Above limit (>10 μg/kg)	Mean value ^a (µg/kg)		
Ground red peppers	25/18	72	23 (0.04-3.47) ^b	2(12.09- 18.68)	2.30		
Walnuts without shell	25/16	64	15(0.66-2.62)	1(10.26)	1.68		
Seedless black raisins	25/16	64	16(0.02-2.07)	-	0.40		
Dried figs	45/23	51	22 (0.16-5.20)	-	1.78		
Total	120/73	68	69(0.02-3.47)	3(5.30-18.68)			

^aAverage contamination on positive samples that higher than the LOD; ^bTotal aflatoxin range.

Table 3 demonstrates the results of the analysis of AFB1 where there are 34 contaminated specimens at a rate $<5 \ \mu g/kg$, 3 specimens varying between 5 and 10.49 $\ \mu g/kg$. As one can see in Table 3, the mean values of ground red peppers, walnuts without shell, dried figs and seedless black raisins are 1.38 $\ \mu g/kg$, 0.86 $\ \mu g/kg$, 0.11 $\ \mu g/kg$, 1.08 $\ \mu g/kg$, respectively. AFB1 was observed only in 37 specimens (38%), in addition to 34 not exceeding the established limit and 3 specimens above the established limit.

	Contami				
Commodities	Number of specimens analyzed /positive	Frequency (%)	Below limit (<5-8*µg/kg)	Above limit (>5-8*µg/kg)	Mean value ^a (µg/kg)
Ground red peppers	25/8	32	6(0.08-1.96) ^b	2(7.41-10.49)	1.38
Walnuts without shell	25/5	20	5(0.66-2.62)	1(5.06)	0.86
Seedless black raisins	25/16	64	16(0.02-0.26)	-	0.11
Dried figs	40/8	20	7(0.29-3.60)	-	1.08
Total	120/37	38	34(0.02-3.60)	3(5.06-10.49)	

Table 3. Aflatoxin B1 in the specimens.

^aAverage contamination on positive samples that higher than the LOD; ^bAflatoxin B1 range.

*Aflatoxin B1 maximum limits for dried fruits in Turkey

Two ground red pepper specimens (12.09-18.68 μ g/kg) exceed the established limit (10 $\mu g/kg$) specified by the Turkish regulations for AFT (TFC 2011), whereas 18 specimens vary between 0.085 and 3.47 $\mu g/kg$ with aflatoxins below the established limit. The presence of AFs in ground red peppers has been documented by numerous researchers from various countries (JUAN et al., 2008). In Turkey, ground red peppers contaminated with AFB1 are 5 to 25 μ g/kg in Bursa and 0.025 to 40.9 μ g/kg in Istanbul, respectively (DOKUZLU et al., 2001). Red pepper specimens contaminated with AFB1 are 1.48 to 70.05 μ g/kg in Kayseri (REDDY *et al.*, 2001). 18.2% of ground red peppers contaminated with total aflatoxins (AFT) varies between 1.1 and 97.5 μ g/kg in Şanlıurfa (ERDOGAN, 2004). SET and ERKMEN (2010) report the contamination rates of AFT and AFB1 ranging from 0.13 to 57.3 μ g/kg and from 0.07 to 55.90 μ g/kg, respectively, in unpacked ground red peppers and from 0.08 to 2.06 μ g/kg and from 0.08 to 1.95 μ g/kg, respectively, in packed specimens. ÖZKAN et al., (2015) state the content of AFB1 exceeds the legal limit in 49 of 180 red chilli pepper specimens and AFs exceeds the legal limit in 37 specimens. FAZEKAS et al., (2005) point to the presence of AFB1 in 25.7% (18/70) of ground red peppers in Hungary. ABDULKADAR et al., (2000) also suggest that 66.7% (4/6) chilli pepper powder contains AFT from 5.60 to 69.28 μ g/kg in Qatar. ROMAGNOLI et al., (2007) observe the AFT contamination in 45.5% red pepper specimens varying between 0.57 and 30.7 μ g/kg in Italy. REDDY (2001) also reports AFB1 contamination of 39.5% of pepper specimens in India ranging from 10 to 99 μ g/kg. The present data indicate that the quality of peppers is better in comparison with the previous studies.

Among the 25 walnuts without shell, only one specimen is contaminated with aflatoxins rate of 10.26 μ g/kg AFT and 5.06 μ g/kg AFB1, which is above the legal limit. Various investigators also have reported similar results. For example LUTFULLAH and HUSSAIN, (2011) report that three in walnuts without shell contain concentrations varying between 6.0 and 10.8 μ g/kg, which exceeds the recommended limit for AFT. GÜRSES, (2006) detected that 6 of 24 walnuts are positive within the contamination range 3-28 μ g/kg. In a

study Mekkah determined quantitative AFB1 and AFT in walnuts 0-17.4 μ g/kg and 0.9-36.6 μ g/kg, respectively (EL TAWILA *et al.*, 2013). ASGHAR *et al.*, (2017) state about 37% walnut specimens showing AFs contamination, ranged from 0.68–6.66 μ g/kg. In another study the contamination levels in walnut specimens vary between 0.56 and 2500 μ g/kg for AFB1 and between 1.24 and 4320 μ g/kg for AFT (JUAN *et al.*, 2008). These values exceed the results presented in this paper.

The AFT rate in the contaminated specimens of dried figs varies between 0.16 and 5.20 $\mu g/kg$, which is below the recommended legal limit. The findings indicate that the AFs contamination of the specimens of the dried figs is low according to many studies as follows. Of late, the aflatoxin contamination level of the dried figs in Turkey has varied between 117.9 to 471.9 $\mu g/kg$ (KARACA and NAS, 2006). KAYA and TOSUN, (2013) state that the aflatoxin rates in dried figs varied between 0 and 10.47 $\mu g/kg$ in 2013. BIRCAN (2009) reports that 34% of the specimens has noticeable levels of AFT (0.20-208.75 $\mu g/kg$) in 7326 dried figs in Aydin province. LUTFULLAH and HUSSAIN, (2011) also point out that the contamination level of AFT in dried fig specimens varies between 0.8 and 12.5 $\mu g/kg$. The values in this study are below these findings. On the other hand, ASGHAR *et al.*, (2017) indicate about 30% samples were contaminated with AFs, ranged from 0.69–3.44 $\mu g/kg$. The results from current study are similar with these previous findings.

In seedless black raisins, all contaminated specimens have lower limits than the maximum rate set by the Turkish regulations (TFC 2011). AFB1 rates detected in all dried figs and the seedless black raisins do not exceed the Turkish acceptable maximum rates ($<8 \mu g/kg$). Despite the low rates of AFB1 observed in most specimens, it still brings with it detrimental risks of health conditions to people who consume those contaminated foods (REDDY *et al.*, 2011). LUTFULLAH and HUSSAIN (2011) report that one of the two contaminated specimens exceeds the recommended rate of aflatoxin while the other is below the recommended rate. 16% of the dried grapes in Brazil was found to contain AFB1 and AFB2 (IAMANAKA *et al.*, 2007).

Several studies have been stated that dried raisins do not exhibit the satisfactory surface or environment for Aflatoxins production. However, ASGHAR *et al.*, (2017) determined that 12 (13%) samples out of 90 tested specimens were determined positive with AFs. The concentrations ranged from 0.24-4.86 μ g/kg. ALGHALIBI et *al.*, (2008) also report that the aflatoxin contamination range of 3 out of 7 dried grape specimens in Sana'a City, Republic of Yemen is between 2678.66 and 11556.88 ng/kg. Whereas in the present study, small quantity of AFT and AFB1 was described in dried seedless black raisins. The concentrations ranged from 0.02-2.07 μ g/kg and 0.02-0.26 μ g/kg respectively. The results from current study indicate that the AFB1 and AFT rates of seedless black raisins from Sakarya city does not exceed the recommended limit by TFC (TFC 2011). In present research the lowest AFB1 and AFT mean level detected in seedless black raisins was 0.11-0.40 μ g/kg respectively. In contrast, ground red peppers exhibited equivalent to 1.30-2.30 μ g/kg for AFB1 and AFT, respectively.

Due to serious toxicity concerned with afaltoxins, various countries established guidelines for the acceptance level of AFs in dry fruits and nuts. For example, the European commission has set the maximum legal limits 4-10 μ g/kg for AFT in dry fruits and nuts, respectively. European maximum limits are 5 and 2 μ g/kg for AFB1 in hazelnuts and dried figs for AFB1, respectively. (EC 2010). Additionally Turkish regulations for aflatoxins consider different limits for unprocessed products and product intended for direct human consumption, as well as for different commodities. For instance TFC has set the maximum legal limits 5-10 μ g/kg in nuts and spices; 8-10 μ g/kg in dried fruits; 2-4 μ g/kg in cereals for AFB1 and AFT, respectively (TFC 2011). In both Turkey (TFC 2011) and the European Commission (EC 2010), the maximum legal limits for ground red pepper are 5 μ g/kg for AFB1 and 10 μ g/kg for AFT. As a result, the determination of AFs showed that there is a powerful need for further research, routine analysis and to establish a monitoring program or project as per food quality control standard and procedures. Furthermore to improve and implement some food quality control procedures and standards such as good manufacturing practices (GMP) and the hazard analysis and critical control point (HACCP) system to minimize the risk and finally prevent the formation of AFs.

4. CONCLUSIONS

The goals of this study are to specify the AFT and AFB1 contamination rates in ground red peppers, dried figs, walnuts without shell and seedless black raisins, and to draw attention to the effects of contamination in these foods on public health. The results of this study provide insight regarding the danger of AFs contained various types of foods. The analysis of all the findings indicates that 68% and 38% of the specimens are contaminated by AFT and AFB1, respectively. Of all the contaminated specimens, 2.5% of specimens exceed the recommended AFB1 (5 μ g/kg) limit defined by the Turkish Food Codex (TFC) regulations. Consequently, aflatoxins entail a health risk for those who consume contaminated foods. Cases of AFs contamination show that constant surveillance and a better food safety system should be implemented so that the content of AFs in foods can be kept at the minimum level (LEONG et al., 2010). On the other hand, more comprehensive studies should be carried out on a wide range of foods consumed in Turkey in order to design appropriate executive projects and to bring forth regulations on AFs encompassing all sustenance consumed. This research will pave the way for new studies on AFs in order to establish a comprehensive food safety system, which will protect humans' health. This study is the primary documentation, which addresses the occurrence of commercial seedless black raisins contaminated with aflatoxin in Sakarya.

ACKNOWLEDGEMENTS

This work was supported by Research Fund of The Sakarya University (Project number: 201-01-16-011).

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Paper Received November 5, 2016 Accepted June 12, 2017