SHORT COMUNICATION

DETERMITION OF AFLATOXIN CONTAMINATION IN SOME DRIED NUTS AND SPICES BY ELISA

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Abstract

This study aimed to determine the total aflatoxin levels in some dried nuts and spices by ELISA (Enzyme-linked Immunosorband Assay). In this study, 1158 some dried and spices samples (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) were randomly obtained from markets and spices shops in Istanbul. Total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (51.96%) of 179 pistachio, in 107 (43.85%) of 244 almond, in 23 (55.95%) of 52 walnut, in 23 (44.23%) of 52 peanut, in 2 (100%) of 2 mahlep, in 7 (78.78%) of 9 sahlep, in 6 (75%) of 8 cinnamon and in 6 (75%) of 8 black pepper. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.

***Keywords*:** aflatoxin, nuts, ELISA.

Introduction

Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (Ayçiçek et al. 2005). Aflatoxins are secondary metabolites produced by species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins easily occur on feeds and foods during growth, harvest or storage (Çolak et al., 2006).

A review of monitoring studies on the occurrence of aflatoxins in food products has demonstrated that aflatoxins are still being found frequently in food products at levels that are of significant concern for consumer protection (Chun et al., 2007).

The European Commission and Turkish government have set limits for maximum levels of total aflatoxin allowed in groundnuts, nuts, dried fruit and their products. For foods ready for retail sale, these limits are 4 $\mu g kg^{-1}$ (total aflatoxins), and for nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs the limits stand at 10 $\mu g\ kg^{-1}$ (total aflatoxins) (Anonymous, 2002a).

ELISA is widely used for analysis at the field of clinical chemistry, veterinary and food control. ELISA analysis is convenient for simultaneous determination of contaminants in a large number of samples with relatively low cost and short time (Chun et al., 2007).

Mostly, spices are grown in tropical and subtropical regions and harvested in poor sanitary conditions. These improper conditions are convenient for the biosynthesis of aflatoxins. Therefore, growing conditions, harvesting and processing methods, storage conditions and postharvest treatments should be carefully controlled in order to prevent aflatoxin risks due to contaminated spices. In addition, training programs should be presented for producers.

Turkey is the world's second largest nut producer, with about 25% of the total world output. The major nuts that are produced in Turkey are hazelnuts, pistachio nuts, almonds, peanuts and walnuts. Turkey is the world's leading hazelnut producer and exporter followed by Italy and the USA and dominates 80% of world hazelnut trade and export process (Exporters Union, 2006). Most of the supply of approximately 210 000 tons per annum is exported to European Union (EU) countries and elsewhere (Exporters Union, 2006). Turkey is also the third main producer of pistachio nuts with 20% of the world production and takes its position among the first 10 countries in almond production. Nuts may be contaminated and invaded by fungi during their development in the field, during transport or storage, and fungal contamination of nuts may result in the production of mycotoxins (Arrus et al., 2005). Higher contamination risk is expected in those that are being transported for long distances and stored for a long time under unhygienic, unventilated, hot and humid conditions (Basaran, Ozcan, 2009).

The study on the total aflatoxin in some dried nuts and spices is important to ensure the safety of nuts and spices for human consumption. The aim of this study was to determine total aflatoxin concentrations in some dried nuts and spices that some of them were exported to Europe and other countries.

Materials and Methods

A total of 1158 samples of dried nuts and spices (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) purchased in Istanbul were randomly obtained from markets and spices shops. Samples were stored at 4 °C in plastic bags until the analysis. All samples were analyzed in duplicate and total aflatoxin concentrations were determined by ELISA. According to Ridascreen Aflatoxin Total (Art No.: 4701) test kit manual, 50 µL of the standard solutions or prepared sample in duplicate were added to the wells of micro-titer plate. Then 50 µL of the diluted enzyme conjugate and 50 µL of the diluted antibody solution were added to each well. The solution was mixed gently and incubated for 30 min at room temperature (20–25 °C)

in the dark. The unbound conjugate was removed during washing for three times (ELISA Washer ELX 50, Bio-tek Inst.). Afterwards, 100 µL of substrate/chromogen solution was added to each well, mixed gently and incubated for 30 min at room temperature (20–25 °C) in the dark. Then, 100 µL of the stop solution (1 M H_2SO_4) was added to each well and the absorbance was measured at 450 nm in ELISA plate reader (ELX 800, Bio-tek Inst.). The mean lower detection limit is 0.25 µg kg^{-1} (Çolak et al., 2006; Anonymous 2002b).

Results and Discussion

During the past decades a huge number of scientific papers have demonstrated that the list of raw materials and processed foods actually contaminated by aflatoxins is continuously increasing spanning from peanuts, known to be contaminated by aflatoxins since 60 s, to cereals, coffee, cocoa, dried fruits and spices (Zinedine et al., 2006). Turkey is a leader in the hazelnut production. And hazelnut and its products are among major exported goods of Turkey. Pistachio is important products that have a high commercial value following hazelnuts (Aluç et al., 2005).

In this study, total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (30%) of 179 pistachio, in 107 (30%) of 244 almond, in 23 (30%) of 52 walnut, in 23 (30%) of 52 peanut, in 2 (30%) of 2 mahlep, in 7(30%) of 9 sahlep, in 6 (30%) of 8 cinnamon and in 6 (97.5%) of 8 black pepper (Table 1). Total aflatoxin level in one of the walnut (1 of 52) and one of pistachio (1 of 179) samples were found higher than the Turkish and EU legal limit (10 µg kg^{-1}) (Anonymous, 2002a) (Table 2).

Yildirim et al.(1997) found total aflatoxins in 8 out of 34 red pepper samples (23.5%) in the range of 1.6–15.0 µg kg^{-1}.

Table 1

Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts

Total Aflatoxin	Hazelnut	Pistachio	Almond	Peanut
Not detected	357 70.00%	86 48.04%	137 56.15%	29 55.77%
>0–0.5 ppb	3 0.30%	7	8	–
>0.5–1 ppb	126 24.50%	48	64	20
>1–2 ppb	24 5.00%	33	26	2
>2–3 ppb	1 0.01%	1	4	1
>3ppb	2 0.01%	4	5	–
Detected samples	156 30.00%	93 51.96%	107 43.85%	23 44.23%
Total	513	179	244	52

In another study performed by Erdogan (2004), it was reported that total aflatoxins was found in 8 red-scaled pepper samples (18.2%) and in 3 red pepper samples (10.7%). Our results were found to be higher than these results. On the other hand, Hazır and Çoksöyler (1998) reported that 46 out of 141 red pepper samples (32.6%) contained aflatoxin. In a similar study in Van, Ağaoğlu (1999) found the highest aflatoxin contamination level of 44.0 µg kg^{-1} in red-scaled pepper.

Aluç, Aluç (2005) checked 367 samples of pistachio produced in 3 year (2002–2004) and it has emerged that 33 samples exceeded the maximum tolerated level in Turkish and European countries (4 ppb). Total aflatoxin was not detected in 75% of pistachio samples. These results are correlated well with our findings.

Abdulkadar et al. (2004) examined total aflatoxin levels in nut from Qatar and found in 23.4% of nut samples with range of 0.53–289 µg kg^{-1}. Aflatoxin contamination was detected in pistachios and peanuts, while other nuts such as almond, cashew nut, walnut and hazel nut were found free from aflatoxins.

In walnut samples, the incidence of AFB1 and AFT was 30%. The contamination levels in walnut samples ranged from 0.56 to 2500 lg kg^{-1} for AFB1 and from 1.24 to 4320 lg kg^{-1} for AFT, respectively. The average contamination levels of walnut with AFB1 and AFT were 360 and 730 lg kg, respectively (Juan et al., 2007). Out of samples, 20% exceeded the maximum limit (2 lg kg^{-1}) fixed for AFB1 by European legislations (European Commission, 2006). Our results are in agreement with the above findings.

Table 2

Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts and spices

Total Aflatoxin	Walnut	Sahlep	Cinnamon	Black pepper
Not detected	63 44.05%	2 22.20%	2 25.00%	2 25.00%
>0–0.5 ppb	1	–	–	–
>0.5–1 ppb	27	3 33.30%	6 75.00%	6 75.00%
>1–2 ppb	21	4 44.50%	–	–
>2–3 ppb	3	–	–	–
>3ppb	28	–	–	–
Detected samples	80 55.95%	7 78.8%	6 75.00%	6 75.00%
Total	143	9	8	8

In conclusion, aflatoxins continue to pose a health concern via human exposure to contaminated spices and nuts. Aflatoxins cause economic and trade problems at almost every stage of marketing of nuts and spices especially during export. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.

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