

SHORT COMMUNICATION

DETERMINATION OF AFLATOXIN LEVELS IN CASHEWS ON TURKISH MARKETS

Ismail Yilmaz¹, Mehmet Aluc²¹ Namık Kemal University, Agriculture Faculty, Department of Food Engineering, Campus Street, No. 1, Tekirdağ, Turkey, e-mail: iyilmaz@nku.edu.tr² Karin foods, Çevrik Mahallesi, Fatih Caddesi No. 5, Cumayeri/Düzce, Turkey**Abstract**

The problem of food contamination with aflatoxin is one of the current concerns and has received a great deal of attention during the last three decades. Aflatoxins are a group of highly toxic secondary metabolic products as some *Aspergillus* species. Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species and humans. They are considered to be one of the most important food contaminants affecting food safety and public health.

The aim of the study is to determine the level of aflatoxins in cashews on Turkish markets. Samples were analyzed by reverse phase HPLC containing post column derivatization and fluorescence detection after immunoaffinity column clean-up. AOAC (999.07) was used in as the methodology of the study.

In this study, aflatoxin B1 and total aflatoxins (B1+B2+G1+G2) were analyzed in totally 50 samples of cashews. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxins were found in 14 out of 50 samples (28%) of cashews (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb). All positive samples did not exceed the maximum limit of 2 µg kg⁻¹ set by EU regulations for aflatoxin B1 and total aflatoxin (4 µg kg⁻¹). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

Keywords: aflatoxin, cashew, HPLC.

Introduction

The cashew tree *Anacardium occidentale* (French.: acajou, anacarde; Spanish.: marañon, caju, acaju) originates from South and Central America (from Brazil to Mexico). The cashew apple is a pseudocarp, and, in botanical terms, is the thickened stem of a fruit which the actual fruit, the cashew nut, is attached. In its originating countries, the pear-shaped cashew apple is eaten fresh. The fruit is distributed throughout the tropics in this form. It was the development of a roasting method to extract the oil from the shell which turned the nut itself into the main product. Cashew nuts are dried before being sold. After the harvest, cashew nuts need to be immediately placed out in the sun to dry, whilst being continually raked over, until the nuts rattle around in their shells (3% rest moisture). In this form, cashew nuts can be stored for up to 2 years, in ideal storage conditions (dry, dark, cool, well-ventilated). Yet they are usually processed within the same year of harvesting (Anonymous, 2000).

Generally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of aflatoxins. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of aflatoxin contamination (Bhat, Vasanthi, 2003).

Mycotoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause diseases or death in humans and animals have a significant economic impact worldwide. *Aspergillus* is one of the most important large genus genera. More than twenty five genes are involved in pathway synthesized aflatoxins (Pearson et al., 1999).

The most toxic of this group, is the most potent carcinogen known. Because of their high toxicity, the presence of aflatoxins in food commodities is believed to pose a risk to human health (Leszczynska et al., 2000). Aflatoxin B1 (AFB1) is considered as the most dangerous toxic metabolite because of its hepatotoxic, teratogenic, immunosuppressive and mutagenic nature. Aflatoxins are regulated in more than 75 countries. Currently, the worldwide range of limits for AFB1 and total aflatoxins are 1–20 ng g⁻¹ and 0–35 ng g⁻¹, respectively (FAO, 2004). European Commission regulations set limits for AFB1 and total aflatoxins of 2 and 4 µg kg⁻¹, respectively in groundnuts, nuts, dried fruit and cereals since 1998 (Commission Regulation, 2006).

Milhome et al. (2014) reported that 24 samples (34.3%), among 70 samples analyzed, were detected presence of total aflatoxins. However, only two samples (2.8%) showed contamination levels above limit set by EU regulations (4.0 µg kg⁻¹), during 2010–2012 period. Aflatoxin contaminations ranging from 2 to 4 mg kg⁻¹ were detected in 22 samples analyzed in 2010–2012, however these samples are in accordance with national and international standards. The incidence of aflatoxins above the allowed limit (EU) has decreased in the last years. In the last year of monitoring (2012) no sample was detected high levels (>4.0 µg kg⁻¹) of total aflatoxins (Milhome et al., 2014).

However, few studies cite the incidence of aflatoxin contamination in cashew nuts. The main objective of this study was to investigate the occurrence and distribution of aflatoxin contamination in cashew nuts imported from India and other tropical countries to assess whether levels of these aflatoxins are affecting food safety and public health.

Materials and Methods

A total of 50 samples of cashews commercialized in Istanbul were randomly obtained from markets and bazaars. Samples were stored at 4 °C in plastic bags until the analysis. All samples were analyzed in duplicate.

Aflatoxins were analyzed in cashew nuts according to the method described by AOAC (2007) first aflatoxin standard solution (containing 1000 ng B1, 200 ng B2, 1000 ng G1 and 200 ng G2 per mL) were prepared in toluene-acetonitrile (98+2). Working standard solutions were prepared daily from these standard solutions according to Stroka et al. (2000) For the extraction procedure, 50 g of sample was added with 5.0 g of NaCl, extracted 300 mL methanol: water (80:20 v/v) in a blender at high speed for 3 min and filtered through a Whatman filter paper No.4. 10 mL were diluted with 60 mL of phosphate buffered saline (PBS), and 66 mL of the diluted filtrate was applied to the immunoaffinity column (Aflaprep, R-Biopharm) previously conditioned with 10 mL of PBS (flow rate of about 3 mL min⁻¹). The column was washed with 15 mL of water and air was drawn through the column until dry. Aflatoxins were eluted by applying 1.25 mL of methanol to the column. The eluate was diluted with 1.75 mL of water. A 100 µL aliquot was injected onto the HPLC system (Hewlett Packard 110 HPLC Chromatograph, equipped with a Hewlett Packard 1100 fluorescence detector). Excitation and emission wavelengths were set at 333 and 460 nm, respectively. The eluate passed through a C18 column (5 µm particle size, 250 mm×4.6 mm). The mobile phase was acetonitrile: water : methanol (17:54:29 v/v/v), and the flow rate was set at 1 mL min⁻¹. The limit of detection of the method was 0.02 µg kg⁻¹. Quantification of each toxin was performed by measuring peak areas at their retention times, and by comparing them with their relevant standard calibration curve. The identity of each toxin was confirmed in all the analyzed samples by injecting sequentially sample extracts and comparing the peak area ratio with their corresponding standard (AOAC, 2007).

Results and Discussion

Due to the contamination of aflatoxins, the nut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide but in India, the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conducive for fungal growth, proliferation and aflatoxin contamination (Reddy et al. 2011). In Turkey, cashews are imported from India and other tropical countries.

The occurrence of aflatoxins in different types of nuts and nutty products has been reported by several authors from different countries. Chun et al. (2007) found that nut samples were contaminated with aflatoxins (10.6% of incidence) in the range of 0.20–28.2 µg kg⁻¹ in South Korea. In China, peanut was found contaminated with aflatoxins, being on the average level of 80.3 µg kg⁻¹

and the highest level being 437 µg kg⁻¹ (Wang, Liu, 2006). In Turkey, Yentür et al. (2006) reported that AFB1 and total aflatoxins in peanut butter were in the range of 2.06–63.7 ng g⁻¹ and 8.16–75.7 ng g⁻¹, respectively. According to Cheraghali et al. (2007), 11.8% and 7.5% of 10,068 Iran pistachio nut samples were above the maximum tolerated level of AFB1 and total aflatoxins, respectively. 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. The incidence and levels of aflatoxins found in this study were relatively low compared to those quoted in the literature.

Table 1

Aflatoxin (B1) and total aflatoxin (B1, B2, G1, and G2) contents of cashew samples

	AFB1	Total Aflatoxin
Not detected	36 (72%)	36 (72%)
>0.5 ppb	14 (28%)	–
>0.5–1 ppb	–	14(28%)
>1–2 ppb	–	–
Detected samples	14 (28%)	14 (28%)
Total	50	50

The present study on assessment of aflatoxin contamination in total cashew nuts has been satisfactorily performed by HPLC. In this study, aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) were analyzed in totally 50 samples of cashew. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxin was found in 14 out of 50 samples (28%) of cashew (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb) (Table 1). Any of the positive samples did not exceed the maximum limit of 2 µg kg⁻¹ set by EU regulations for AFB1 and AFT (4 µg kg⁻¹). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

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