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Aflatoxin contamination in tahini

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Received: 8 February 2012 / Accepted: 23 May 2012

RESEARCH PAPER

Abstract

Sesame seeds can be contaminated with aflatoxins during growth or storage. Therefore, tahini, a sesame seed-based product, has potential risk of aflatoxin contamination. In this study, a total of 104 tahini samples collected from Central Anatolian region of Turkey were analysed for aflatoxin contamination. Analysis of samples was carried out by high-performance liquid chromatography with fluorescence detection after immunoaffinity column clean-up, with recoveries ranging from 70.3 to 82.4%. In terms of incidence and quantity, aflatoxin B_1 was the predominant mycotoxin detected in 15 samples, with contamination levels ranging from 0.31 to 2.53 µg/kg. The mean level of total aflatoxins in contaminated samples was found to be 1.17±0.55 µg/kg. Our data revealed that aflatoxin B_1 and total aflatoxins levels determined in tahini samples were within the Turkish Food Codex limits. The incidence of contaminated tahini samples indicated that a regular surveillance program should be established to monitor the occurrence of aflatoxins in tahini.

Keywords: aflatoxin, HPLC, sesame seed-based product, Tahini

1. Introduction

Tahini, also called sesame paste, produced by milling from hulled and roasted sesame seeds, is a popular food in Middle Eastern and eastern Mediterranean countries and is used as an ingredient in many kinds of food (El-Adawy and Mansour, 2000; Lokumcu and Ak, 2005). Tahini has high nutritional value because it is rich in lipid, protein, dietary fiber, niacin, thiamin and some minerals such as calcium, iron and phosphorus (Akbulut and Çoklar, 2008).

Sesame seeds are sensitive to invasion of aflatoxin-producing moulds and accumulation of aflatoxins (Reddy *et al*., 2011). Several studies showed that aflatoxins can be found in sesame seed-based products such as sesame seed oil, tahini and tahini halva (Nilüfer and Boyacıoğlu, 2002; İdris *et al*., 2010; Li *et al*., 2009; Var *et al*., 2007).

Aflatoxins is a group of toxic metabolites produced by certain species of *Aspergillus*, classified as group 1 carcinogens by the International Agency of Research on Cancer, primarily affecting the liver (IARC, 2002). Besides their carcinogenic effects on the human body, aflatoxins also cause malabsorption syndrome and decreased bone strength (Nelson *et al*., 1982; Osborne *et al*., 1982). The common aflatoxins are B_1, B_2, G_1 and G_2. Among these, aflatoxin B_1 is the most toxic, followed by aflatoxin G_1 (Cho *et al*., 2008).

To date, there are limited published studies indented for aflatoxin contamination in tahini. Consequently, the aim of this study was to investigate aflatoxin contamination in tahini produced and consumed in Turkey by immunoaffinity clean-up and high performance liquid chromatography (HPLC) technique.

2. Materials and methods

Samples

A total of 104 tahini samples were collected within their original packages from different retail outlets and producers in the Central Anatolian region of Turkey and stored at 4 °C until analysis. According to their label information, all samples analysed in this study were produced from roasted sesame seeds.

Chemicals

Liquid chromatography grade methanol, acetonitrile, water and reagent grade NaCl, KBr, HNO_3 (65%) were purchased from Merck (Darmstadt, Germany). The mixed aflatoxin standard stock solution containing aflatoxin B_1 and aflatoxin B_2 at 1000 ng/ml, and aflatoxin B_2 and aflatoxin G_2 at 300 ng/ml in methanol was purchased from Supelco (Bellefonte, PA, USA).

Method

Analysis of aflatoxins in tahini samples was performed based on the AOAC official method 991.31 (AOAC, 1994). Prior to the analysis of the samples, the applicability of the method to the specific tahini matrix was demonstrated with spiking experiments.

Calibration curves

Six point calibration curves were constructed before analysis for each of the aflatoxins by using the working standard solutions. These solutions covered the ranges of 0.40-5.40 ng/ml for aflatoxin B_1 and aflatoxin G_1, and 0.12-1.62 ng/ml for aflatoxin B_2 and aflatoxin G_2.

Recovery experiment

A blank tahini sample, which was determined to be free from the aflatoxins, was spiked with aflatoxins for the recovery experiment. Spiking was performed by adding 1 ml of a ten-fold dilution of mixed aflatoxin standard stock solution in methanol to 25 g of the blank sample, and allowing the solvent to evaporate. Thus, the blank sample was fortified with aflatoxin B_1 and aflatoxin G_1 at 4.00 µg/kg, and aflatoxin B_2 and aflatoxin G_2 at 1.20 µg/kg.

Extraction and purification of aflatoxins

The sample (25 g) was weighed into a 250 ml flask and 5 g NaCl and 125 ml extraction solvent (methanol-water, 70:30, v/v) were added. The mixture was homogenized with a shaker (SL350; Nüve, Ankara, Turkey) for 30 min and then filtered through fluted filter paper (Vicam, Somerville, MA, USA). The 15 ml filtrate was transferred into a flask and 30 ml water was added. This dilute filtrate was filtered again through a microfiber filter (Vicam) and the 15 ml of final filtrate was used in an immunoaffinity column clean-up.

The filtrate was injected into the immunoaffinity column (Aflatest, Vicam) at a flow rate of 1-2 drops/s and then the column was washed two times with 15 ml water at the same flow rate. Elution of aflatoxins was accomplished by passing 1 ml of methanol through the column. The eluate was collected in a 2 ml volumetric glass vial and diluted to volume with water.

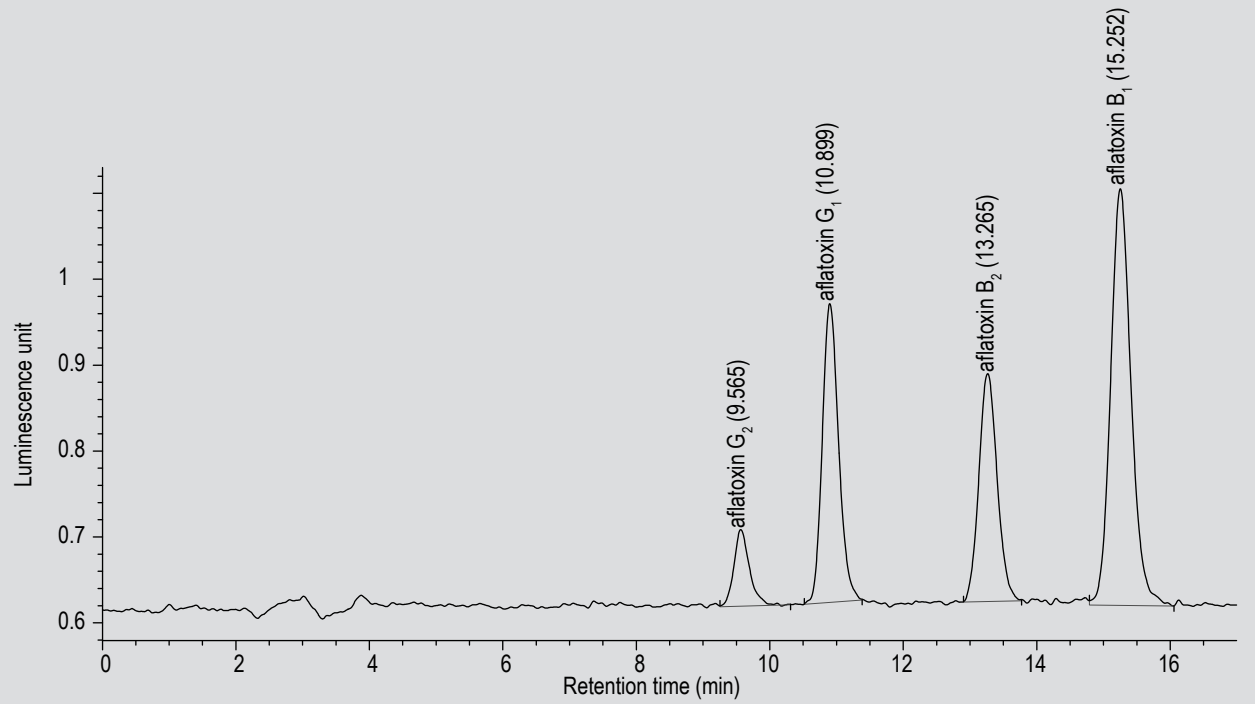

Figure 1. Typical chromatogram of a blank tahini sample spiked with aflatoxin B_1 and aflatoxin G_1 at 4.00 µg/kg, and aflatoxin B_2 and aflatoxin G_2 at 1.20 µg/kg.

HPLC determination of aflatoxins

A HPLC system (1100 series; Agilent, Palo Alto, CA, USA) equipped with a fluorescence detector was used for detection and quantification of aflatoxins. The chromatographic separation was performed on a reversed phase Spherisorb ODS2 C18 column (250x4.6 mm, 5 µm particle size; Hichrom, Reading, UK). The mobile phase was water-acetonitrile-methanol solution (60:20:20, v/v/v), containing 120 mg KBr and 100 µl HNO_3. A post-column derivatization was applied to produce electrochemically generated bromine (Kobra Cell; R-Biopharm Rhone, Glasgow, UK). The fluorescence detector was operated at an excitation wavelength of 360 nm and emission wavelength of 430 nm. Aflatoxins were identified by the retention times. Typical retention times for aflatoxin G_2, aflatoxin G_1, aflatoxin B_2 and aflatoxin B_1 were 9.6, 10.9, 13.3, and 15.3 min, respectively (Figure 1). The amounts of aflatoxins in the injected volume (100 µl) were quantified by the peak areas. These amount values were multiplied by the extraction factor of 20 to obtain the level of each of the aflatoxins in the original sample as µg/kg. Recovery correction was applied to the results.

Table 1. Performance characteristics of the analytical method.

Aflatoxin	R^2	LOD[a] (µg/kg)	LOQ[b] (µg/kg)	Recovery (%)	RSD_r[c]
Aflatoxin B_1	0.999	0.1	0.3	82.4	4.8
Aflatoxin B_2	0.999	0.1	0.2	74.8	4.0
Aflatoxin G_1	0.999	0.1	0.3	81.1	5.2
Aflatoxin G_2	0.999	0.1	0.3	70.3	6.1

[a] Limit of detection.
[b] Limit of quantification.
[c] Relative standard deviation of repeatability (n=7).

3. Results and discussion

The HPLC technique was used for detection and quantification of aflatoxins in tahini samples. The method performance characteristics are given in Table 1. The trueness and precision were evaluated by multiple analyses of a blank tahini sample spiked at 4.00 µg/kg for both aflatoxin B_1 and aflatoxin G_1, and 1.20 µg/kg for both aflatoxin B_2 and aflatoxin G_2. The mean recoveries and relative standard deviations of repeatability of aflatoxins were in the range of 70.3-82.4% and 4.0-6.1%, respectively. These values are within the acceptable method performance limits of 1-10 µg/kg according to the Turkish Food Codex directive (TFC, 2011) on mycotoxins analysis in foods. The limit of detection was 0.1 µg/kg for four aflatoxins. The limit of quantification was 0.3 µg/kg for aflatoxin B_1, aflatoxin G_1 and aflatoxin G_2, and 0.2 µg/kg for aflatoxin B_2. Previously, Nilüfer and Boyacıoğlu (2002) reported that the HPLC technique can be effectively employed for the determination of aflatoxin contamination in tahini samples due to its high recoveries and low variance.

The levels of aflatoxins detected in tahini samples are summarized in Table 2. In this study, 16 samples from a total of 104 samples (15%) were found contaminated with aflatoxins in the range of 0.27-3.08 µg/kg. In terms of incidence and quantity, aflatoxin B_1 was the predominant detected in 15 samples, with contamination levels ranging from 0.31 to 2.53 µg/kg. Whereas, aflatoxin G_1 was detected in three samples and aflatoxin G_2 was not detected in any sample. Aflatoxin B_2 was determined in seven samples with contamination levels ranging from 0.27 to 1.07 µg/kg.

The Turkish Food Codex (TFC, 2008) provides maximum allowable limits for total aflatoxins and aflatoxin B_1 levels in tahini of 10 and 5 µg/kg, respectively. Our data revealed that the contamination levels found in all of the tahini samples were lower than the Turkish regulatory limits. These results were consistent with other previous surveys performed in Turkey. Nilüfer and Boyacıoğlu (2002) analysed 14 tahini samples randomly obtained from retailers and they

Table 2. Incidence and contamination levels of aflatoxins in tahini samples.

Aflatoxin	Number of positive samples	Mean±SD	Number of samples in the contamination range			
			< LOD[a]	LOD-1 µg/kg	1-5 µg/kg	>5 µg/kg
Aflatoxin B_1	15	0.93±0.62	89	11	4	-
Aflatoxin B_2	7	0.49±0.27	97	6	1	-
Aflatoxin G_1	3	0.46±0.17	101	3	-	-
Aflatoxin G_2	-	-	104	-	-	-
Total aflatoxins	16	1.17±0.55	88	11	5	-

[a] Limit of detection.

determined aflatoxin contamination in only one sample. Var *et al*. (2007) evaluated plain tahini halva samples in terms of aflatoxin contamination and no levels were reported. However, Li *et al*. (2009) analysed 100 Chinese tahini and found the aflatoxin B_1 contamination in 32 samples to be higher than the Chinese regulatory limits.

The aflatoxin contamination in tahini samples can be explained by the use of contaminated sesame seeds in their production. The aflatoxin-producing moulds can colonize on sesame seeds and accumulate high level of aflatoxin contamination when environmental conditions are favourable for their growth (Reddy *et al*., 2010). Several surveys conducted in Turkey, Iran, Japan and Cyprus showed that aflatoxins can be found in sesame seeds (Asadi *et al*., 2011; Ioannou-Kakouri *et al*., 1999; Tabata 1993; Ulca *et al*., 2010; Yentür *et al*., 2006).

Roasting of sesame seeds is a basic operation for the production of tahini (Kahyaoğlu and Kaya, 2006). Although aflatoxins are highly stable to dry heat due to their high thermal decomposition temperatures (Betina, 1989), results of several studies showed that aflatoxins in nuts and seeds could be degraded by roasting (Bullerman and Bianchini, 2007). Roasting of sesame seeds during the traditional production of tahini should be performed at 100-150 °C for 2 h (Özcan and Akgül, 1994). To date, no published study has assessed the degradation of aflatoxins during the roasting process in production of tahini. Yazdanpanah *et al*. (2005) reported that roasting of spiked pistachio nuts at 150 °C for 30-120 min reduced the aflatoxins levels in samples by 17-63%. However, these results also indicated that aflatoxins in foods cannot be degraded completely by roasting.

Our results showed that the mean contamination levels of total aflatoxins (1.17±0.55 µg/kg) and aflatoxin B_1 (0.93±0.62 µg/kg) in tahini samples were clearly below Turkish regulatory limits. Therefore, the current status of Turkish tahini in terms of aflatoxin contamination cannot be regarded as a serious risk to public health. Nevertheless, the incidence of contaminated tahini samples verified that the roasting process applied in the tahini production is insufficient for completele degradation of aflatoxins in sesame seeds. For this reason, sesame seeds used for production of tahini should be controlled regularly for aflatoxin contamination. Furthermore, other possible fungal contamination sources in production, packaging and storage processes of tahini should be identified and kept under control.

Acknowledgement

The authors would like to thank Osman Murat Asker, head of the Mycotoxin Department of Konya Food Control Laboratory, for his valuable contributions.

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