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Determination of Aflatoxin Levels in Consumed Hazelnut Cream in Istanbul, Turkey

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This study was carried out to determine aflatoxin levels in cacao hazelnut cream, which is a widely consumed product. First, a total of 182 cacao hazelnut cream samples were obtained randomly from retail points in Istanbul. Then they were analysed from January 2002 to December 2004 by ELISA technique for aflatoxin B_1 and total aflatoksin. 51 Samples (28.02%) were investigated for total aflatoxin and 48 samples (26.37%) were investigated for AFB_1 which were found to have exceeded the highest acceptable limits set down by the EU legal limit. The acceptable limits of the Turkish food codex are different from the EU. Out of 182 samples, only 10 (5.49 %) were found to have exceeded the limits for total aflatoxin and only five (2.74 %) were found to have exceeded the limits for aflatoxin AFB_1 that were set down by the Turkish food codex.

Key Words: Hazelnut cream, Total aflatoxin, Aflatoxin B_1.

INTRODUCTION

The species of hazelnut, genus *Corylus*, are distributed in the northern hemisphere[1,2]. The main hazelnut producing countries are Turkey, Italy, Spain and USA[2-4]. Turkey is the top most hazelnut producing country in the world by producing *ca.* 500.000 tons of hazelnut, annually. Turkey is at the capacity of producing globally about 70 % of the total hazelnut. In addition to this, Turkey exports about 80 % of this production[1,4,5].

The handling of hazelnut after harvest can be described as having two stages. In the first stage, the shell is cracked and the kernel separated. In the second stage, the kernel may undergo a variety of processes namely blanching, roasting, chopping, slicing, shredding, grinding or being made into a paste. Salt, sweeteners and emulsifiers may be added during the production stages[3,4].

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80 % of the hazelnut kernels is used in chocolate production, 15 % in confectionery, biscuit and pastry production and the remaining 5 % is consumed without any further processing. They are used to provide a specific flavour in somefood materials such as dairy, bakery and confectionery products or are used to enhance flavour of chocolate, ice cream, desserts and snack bars[1,3,6].

Hazelnut is extremely high fat commodities, rich in calories because the edible portion of hazelnut has a high protein and polyunsaturated fat content. It is also excellent sources of essentially amino acids, essentially fatty acids, calcium, phosphorus, iron, potassium, magnesium, selenium, vitamins B_1, B_2, B_6 and E. The hazelnut plays a major role for human nutrition, because of its special composition of fat, proteins, carbohydrates, vitamins, minerals, dietary fibre and other nutritients[1-3,7-10].

The aflatoxins, produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* are the most common mycotoxins found in hazelnut and its products[3]. The mycoflora of hazelnut and the presence of aflatoxins was studied by several researchers[11-13].

Hazelnut cream is a popular product and commonly consumed. It may consist many ingredients and flavouring substances like cacao. The aim of this study was to determine the aflatoxin levels of cacao hazelnut cream.

EXPERIMENTAL

A total 182 cacao hazelnut cream spread samples (40, 250, 350 g) were obtained randomly from retail points in Istanbul. They were analyzed from January 2002 to December 2004 and aflatoxin level were determined by microtitre plate Enzyme-Linked Immunosorbent Assay[4] (ELISA) (Ridascreen, Darmstadt, Germany).

Preparation of Samples

The quantitative analysis of AFB_1 and total aflatoxin (Total AF) levels were determined in the hazelnut cream samples. The samples were analysed with the help of procedures called AFB_1 (Art No: R1201) and Total AF (Art No: R4701), which were described by producer company (R-Biopharm GmbH)[14].

The Rida® columns are usable in combination with enzyme immunoassay (Ridascreen total AF and AFB_1). For the sample preparation of oily food (nuts), spices, herb leaves Ridascreen recommends immunoaffinity purification with Rida® Aflatoxin Column (Art. No: R 5001/5002) prior to total AF (Art No: R4701) and AFB_1 (Art No: R1201) test procedure.

10 g of sample and 50 mL methanol (70 %) were mixed by magnetic stirrer (Janke & Kunkel, Germany) for 10 min. The extract was filtered (Whatman no. 1). 5 mL of the filtered solution were added to 15 mL distilled water, then 0.25 mL Tween 20 (Merck, 8.22184) was added to this

solution. The solution was mixed by magnetic stirrer for 2 min. The entire amount of the sample solution (20 mL) was filtered through the column.

Separation with aflatoxin column

The column was rinsed with 2 mL distilled water for equilibration. The column was filled with approximately 1 mL sample extract. A suitable adapter was attached on top of the column and a syringe was used as a sample reservoir. Syringe was filled with the residual sample extract. The sample extract was filtered slowly and continuously through the column (flow rate: *ca.* 1 drop/sec.). The filtered solution was discarded. The column was rinsed with 10 mL distilled water and the filtered solution was discarded. Additionally, column was air-pressed for approximately 10 s, in order to make sure that the entire residual buffer is removed from the column. Syringe was removed and vial placed directly below the column. 0.5 mL methanol (100 %) was filtered slowly through the column (flow rate: *ca.* 1 drop/s). The eluent was filtered faster than 10 s collected and filtered again through the column. The column was air-pressed again for 0.5 min to collect all eluate residues.

Total AF analyses

Toxin containing eluate was diluted (1 + 9) 1:10 with the corresponding sample dilution buffer 1 (phosphate buffer solution (PBS)-buffer, pH 7.2) of the respective test (50 + 450 µL sample dilution buffer). The Total AF standards and test samples (50 µL) in duplicate were added to wells of micro-titer plate precoated with antibodies coated for aflatoxins B_1, B_2, G_1 and G_2. Diluted enzyme conjugate (50 µL) transferred to each well and plate incubated at room temperature (20-25°C) in dark for 0.5 h. After the washing step, 50 µL each substrate (urea peroxide) and chromogen (tetramethlybenzidine) were added to the wells and plate incubated again for 0.5 h at room temperature in the dark. The reaction was stopped by 100 µL 1N H_2SO_4 and the absorbance were measured at 450 nm in microplate ELISA reader (EL × 800 Universal, Biotek, USA).

AFB_1 analysis

The toxin containing eluate (1 + 9) 1:10 diluted with corresponding sample dilution buffer 1. 50 µL aflatoxin standard solutions and 50 µL prepared test samples were added into separate wells of micro-titer plate, in duplicate. Plates were incubated for 2 h at room temperature in the dark. The liquid was then removed completely from the wells, than each well was washed with 250 µL washing buffer (PBS-Tween-Buffer, pH 7.2) and this repeated two more times. Subsequently, enzyme substrate (urea peroxide, 50 µL) and chromogen (tetramethyl-benzidine, 50 µL) were added to each well and incubated for 0.5 h at room temperature in the dark. 100 µL

of the stop reagent (1 M H_2SO_4) was added and the absorbance was measured at 450 nm in ELISA reader.

Evaluation

The mean values of the absorbances for the standards and the samples were evaluated according to the Rida® Soft Win program (Ridavin.exe) distributed by Ridascreen (R-Biopharm). The lower detection limit of the total AF test in the analysis procedure was 0.250 µg/kg. The lower detection limit of the AFB_1 test in the analysis procedure[14] was 0.125 µg/kg, recovery rate was 90 %.

RESULTS AND DISCUSSION

In the areas of traditional productions, the nuts are usually dried out under the sunlight after the harvest and they become so much affected with the humidity of the environment[15]. In further steps such as decoating, peeling, washing and such processes, risk of decaying shows up and aflatoxin is tend to appear in combination with these substances[16]. This risk of aflatoxin in raw nut must always be taken into consideration for public health in Turkey as well as the countries of exportation.

The European Commission[17] and Turkish Food Codex[18] have set limits for maximum levels of total aflatoxin and AFB_1 allowed groundnuts, nuts, dried fruit and their products. 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 4 µg/kg (EU)[17], 10 µg/kg (Turkish Food Codex)[18] for total aflatoxins and 2 µg/kg (EU)[17], 5 µg/kg (Turkish Food Codex)[18] for AFB_1.

The occurrence of Total AF and AFB_1 in hazelnut cream is shown in Tables 1 and 2. A total of 182 samples were investigated for this research. Out of 182 samples, 51 samples (28.02 %) were detected with an exceed of Total AF and 48 samples (26.37 %) were detected with an exceed of AFB1. These were found to have exceeded the levels set down by the EU legal limits[17]. Furthermore; 10 samples (5.49 %) analyzed for Total AF and 5 samples (2.74 %) analysed for AFB1 were found to have exceeded levels set down by the Turkish Food Codex[18]. The highest Total AF level in the hazelnut cream samples was 51 µg/kg and AFB1 level was 11 µg/kg.

Ayçiçek *et al.*[13] examined aflatoxin level in 40 cacao hazelnut cream samples in Ankara, Turkey between year 2002 and 2003. AFB_1 contamination was detected in 38 (95 %) samples of cacao hazelnut cream (ranging from < 1 to 10 µg/kg). Total AF contamination was determined in 39 (97.5 %) samples of cacao hazelnut cream. Total AF levels in only 1 out of 40 hazelnut cacao cream samples were determined to have exceeded the limits set down by the Turkish Food Codex[18] allowed. Günsen and Büyükyörük[19] detected AFB_1 contamination in 19 out of 25 cacao hazelnut

cream samples. The AFB_1 level was found in the range 0-3725 mg/kg (1076.5 ± 194.4 μg/kg). The present results seem to be in accordance with the reported results.

TABLE-1
TOTAL AF LEVELS IN HAZELNUT CREAM SPREAD PRODUCT

Aflatoxin levels (Total no. of 182 samples)	Total AF
	Sample (%)
≤ 0.250 μg/kg (Min. detection limit)	28 (15.39)
0.251 – 1 μg/kg	64 (35.16)
1.1 – 4 μg/kg	39 (21.43)
4.1 – 10 μg/kg[a]	41 (22.53)
> 10 μg/kg[b]	10 (5.49)

[a]Exceed EU maximum tolerable limits for Total AF
[b]Exceed Turkish Food Codex maximum tolerable limits for Total AF

TABLE-2
AFB_1 LEVELS IN HAZELNUT CREAM SPREAD PRODUCT

Aflatoxin levels (Total no. of 182 samples)	AFB_1
	Sample (%)
≤ 0.125 μg/kg (Min. detection limit)	27 (14.84)
0.126 – 1 μg/kg	95 (52.20)
1.1 – 2 μg/kg	12 (6.59)
2.1 – 5 μg/kg[a]	43 (23.63)
> 5 μg/kg[b]	5 (2.74)

[a]Exceed EU maximum tolerable limits for Aflatoxin B_1
[b]Exceed Turkish Food Codex maximum tolerable limits for Aflatoxin B_1

Abdulkadar *et al.*[20] analyzed edible nuts for aflatoxins contamination in Qatar between 1997 and 1998. Total 81 nut samples were analyzed. Contaminant aflatoxins in 19 samples were found and these aflatoxins varied from 0.53 to 289 μg/kg. Abdel-hafez and Saber[21] examined aflatoxin B_1, B_2, G_1 and G_2 in hazelnut in Egypt and found 90 % of samples with range 25-175 μg/kg.

Several environmental factors are known to influence aflatoxin production, but temperature and relative humidity are considered to be the most critical. Additional factors such as water activity, moisture, storage time and insect damage also influence fungal growth (*A. flavus*, *A. parasiticus*) and aflatoxin production[13,22]. The cause of aflatoxin is observed due to the inconvenience environmental conditions such as the improper storage after the harvest, under high temperatures or humidity and some other non-hygenic conditions. The proper applications (GMP, GHP) before the harvest *i.e.*, safe drying, optimum conservation and disabling

the fungal contamination of the products may decrease the risk of aflatoxin and could be very effective for higher performance and quality.

In conclusion, hygienic rules during production of cacao hazelnut cream should be taken more seriously. More effective measures should be taken during acceptance and storage of hazelnut that will be used in hazelnut cream production.

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