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ORIGINAL ARTICLE

Determination of Aflatoxin in Pistachio samples by ELISA Technique in Torbat-e-Heydarieh (North-east of Iran)

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ABSTRACT
The aim of this study was to detect the amount of aflatoxin total (AFT) in raw pistachio samples in Torbat-e-Heydarieh in north-east of Iran. For this purpose, 48 raw pistachio samples were collected from Karimi farm in Torbat-e-Heydarieh during October 2014 and analyzed for AFT content by enzyme linked immune-sorbent assay (ELISA) technique. Results showed that the the minimum, maximum and average ± standard deviation of AFT was respectively 0.27, 0.3, 0.95±0.62ng/g in the raw pistachio samples. The AFT concentration in the samples was lower than the national (15ng/g) and EU (10ng/g) standards. The situation seems to be suitable in the raw samples, but the storage condition must be in mind to prevent the contamination and production of the mycotoxin.
Keywords: Aflatoxin, Pistachio, ELISA, Torbat-e-Heydarieh.

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INTRODUCTION

Mycotoxins are secondary metabolites of molds which are associated with certain disorders in animals and humans. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer, and it is this aspect which has evoked global concern over feed and food safety (1). The major types of aflatoxins are B1, B2, G1, G2 and M1. Among them aflatoxin B1 is a very potent carcinogen to humans and animals, the main metabolite produced by fungi of the genus *Aspergillus,* particularly *A. flavus, A. parasiticus,* and *A. nomius* (2). The Pistachio nut is a commodities with the highest risk for aflatoxin contamination (3). The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish a maximum tolerable level (MTL) on mycotoxins in food and feedstuffs in the last decades to safeguard the health of humans, as well as the economic interests of producers and traders. Currently, worldwide range for the limits of AFB1 and Aflatoxin total are 1-20 ng/g and 0-35 ng/g, respectively (4). More details about the current standards in different countries are presented in Table 1.

Table1. Summary of aflatoxin regulations (total aflatoxins) for pistachios in selected countries (5)

Country	Standard for total allowable aflatoxins in 1995 (ng/g)	Standard for total allowable aflatoxins in 2003 (ng/g)
Iran	No Regulation	15
USA	15	15
European Union (EU)	No Regulation	4 (Changed to 10 in 2009)
Belgium	No Regulation	4 (Changed to 10 in 2009)
Canada	15	15
Germany	4	4 (Changed to 10 in 2009)
Hong Kong	15	15
Japan	20	20
Saudi Arabia	No Regulation	No Regulation
China	No Regulation	No Regulation
Egypt	No Regulation	No Regulation
The Netherlands	10	4 (Changed to 10 in 2009)
Russia	5	5

Previous studies have shown that the aflatoxin content in exported lots did not meet the required standards (6, 7, and 8). The purpose of this study was to determine the AFT levels in the pistachio samples collected from the pistachio farm of Karimi in Torbat-e-Heydariyeh in Khorasan-Razavi province in Iran.

MATERIALS AND MTHODS

Materials

In this study the AFT content of 48 raw pistachio samples collected in October 2014, from Karimi farm, in Torbat-e-Heydariyeh in Khorasan Razavi in northeast of Iran determined by ELISA technique.

Methods

Samples treatment

Samples were analyzed by the ELISA method. Euro-Diagnostic ELISA test kits (Sweden) was used for analysis. AFT extraction was carried out according to the manufacturer's instructions For this purpose, 10g of grinded samples was taken and 50mL of 33% methanol solution (methanol: distilled water, 30:60) added, shaken for two minutes and then let settle for 15 minutes at room temperature. Then, the extract was filtered through a Whatman No 1 filter paper, the clear supernatant was diluted 1:2 with 33% methanol solution (1mL+1mL) and samples were tested through ELISA kits instructions. The topical density was measured at 450 nm using ELISA plate reader (ELx 800, Bio-Tek USA). Evaluation of the ELISA data as well as the AFT concentration was performed using the software program (Euro-Diagnostic).

Statistical Analysis

SPSS program version 11.5 was used for statistical analysis (SPSS Institute Inc. Chicago, Illinois). Descriptive statistical analysis was reported as mean ± SD.

RESULTS AND DISCUSSION

Results showed that the minimum, maximum and average ± standard deviation of AFT was respectively 0.27, 0.3, 0.95±0.62ng/g in the raw pistachio samples. The distribution of AFT contamination in the samples is presented in Table 2.

Table2. AFT distribution in raw pistachio samples

AFT levels (ng/g)	<0.1 ng/g	0.1-0.3 ng/g	0.3-1 ng/g	>1 ng/g
Number of contaminated samples	34	14	0	0
Percentage of samples containing AFT	71	29	0	0

The situation seems to be suitable in comparison to the other research in this field. The occurrence of AFB_1 in pistachio nuts has been studied by many researchers in the various countries. In Sweden, 9.5% of pistachio nuts contained AFB_1 higher than 2ng/g [9]. A study on 100 pistachio samples purchased from retail stores in Esfahan province in Iran in 2007 revealed that 36% of the samples had AFB_1 exceeded the MTL set for this mycotoxin [10]. Another study by Heraghali et al., [11] showed that pistachio samples produced in Kerman and Rafsanjan in 2002-2003 had contamination in 11.8% of the samples higher than the MTL set in Iran for AFB_1. Dini *et al*., [3] studied on 3181 commercial raw pistachio samples for testing for European export certification between 2009-2011. Results showed that $AFB_1$was detected in 23.4% of the samples with the mean value of 2.18ng/g which was lower than the MTL level set in Iran.

The difference in our study compared to other research is studying raw pistachio samples which were fresh and had not been stored. The main reasons for high AF contamination in pistachio samples are susceptible conditions such as high temperature, relatively high humidity, low light intensity, long-term storage and old and poor methods of manufacturing, transition and marketing.

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