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Aflatoxins in Pistachios Consumed in Mexico

Gavilán-Ruiz JM[1], Carvajal-Moreno M[1*], Rojo-Callejas F[2] and Ruiz-Velasco S[3]

[1]*Mycotoxins Laboratory, Department of Botany, Institute of Biology, National Autonomous University of Mexico (UNAM). 04510 Mexico, DF*

[2]*Department of Analytical Chemistry, Faculty of Chemistry, UNAM. 04510 Mexico, DF*

[3]*Department of Probability and Statistics, Institute of Research in Applied Mathematics and Systems, UNAM. 04510 Mexico, DF*

Abstract

Introduction Aflatoxins (AFs) are fungal secondary metabolites with potent carcinogenic properties´ and pistachios are susceptible oilseeds for AF contamination.

Objective To determine the concentration of the four AFs types (AFB_1, AFB_2, AFG_1 and AFG_2) from 48 pistachio samples from Mexico, six from Turkey and three from Greece.

Methods The AFs of the 57 analysed samples of pistachio seeds were quantified by liquid chromatography. The calibration curves had R^2 values near 1. The LOD values were low and were acceptable to identify and quantify the AFs. The recovery percentages were >80%, which indicated that the extraction method was efficient, and in the selectivity test, no interference between the retention times of the AFs and the pistachio matrix were observed.

Results This study validated an analytical method developed to quantify AFs in pistachios. All samples were contaminated with trace levels of AFs. A total of 36.4% were contaminated with AFB_1 (0.10 to 4.15 ng/g), 100% of the samples had both AFB_2 (0.01 to 4.00 ng/g) and AFG_1 (0.02 to 1.02 ng/g), 56.4% had AFG_2 (0.46 to 8.09 ng/g) and 100% were contaminated with total aflatoxin (AFt) (0.04 to 8.39 ng/g). The samples most contaminated with AFB_1 (4.15 ng/g) and AFG_1 (1.02 ng/g), and with AFB_2 (4.00 ng/g) were from Mexico. The highest contamination by AFG_2 (8.09 ng/g) and AFt (8.39 ng/g) was in one sample from Istanbul, Turkey.

Conclusion All the samples resulted contaminated with AFs although the levels do not risk the health because they are below the tolerance levels of World Health Organization (5 μg kg^{-1} for AFB_1 and 10 μg kg^{-1} for AFt) and Food and Agriculture Organization of United Nations (FAO) (20 μg kg^{-1} for AFB_1 and 35 μg kg^{-1} for AFt).There was a significant difference between the AF content and the place of origin of the samples.

Keywords: Pistachios; Aflatoxins; Contamination; Toxicology; Carcinogens; Mutagens

Introduction

Pistachios are consumed and exported raw, toasted and salted for use in desserts, ice creams, aromatisers, condiments and sweets [1]. The main countries of production are Iran, the United States, Turkey, and China; however, Mexico produced 38 tons and was in the 18th position in 2012 [2]. *Aspergillus flavus, A. parasiticus* [3], *A. pseudotamarii* [4], *A. bombycis* [5], *A. tamarii* [6] and *A. ochraceoroseus* [6] are moulds that produce aflatoxins (AFs), and they have an affinity for oil seeds, including pistachios. AFs are potent mutagens, carcinogens [7] and teratogens [8] that can cause acute effects, called aflatoxicoses, when consumed in mg quantities. These aflatoxicoses have several symptoms, such as bleeding, diarrhoea, liver disease, oedema, digestion alteration, changes in food metabolism and occasionally death [9]. Chronic effects are produced when AFs are consumed in μg or ng amounts, such as low nutrient absorption, slow growth, immunodepression, low response to vaccination, cancer, Reye syndrome, hepatitis, cirrhosis, Kwashiorkor or protein malnutrition and kidney problems. Epidemiological studies from Europe, Africa and Asia indicate that there is a positive correlation between liver cancer and the consumption of AF contaminated foods [10]. AFB_1 is metabolized in the liver, where it is hydroxylated to form aflatoxin P_1 (AFP_1), aflatoxin M_1 (AFM_1) and aflatoxin Q_1 (AFQ_1). AFB_1 can also be oxidated to link with DNA, RNA and proteins as the unstable AFB_1-8, 9-epoxide, which affects cell transcription and translation. When this epoxide links to nitrogen 7 (N^7) of guanine DNA residues, it forms an 8, 9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B_1 (AFB_1-N^7-Gua) adduct. This adduct interferes with transcription and diminishes ribonucleic acid synthesis, and it can produce a mutation or initialise cancer [10]. Moreover, the liver is the organ that forms and accumulates most of the AFB_1-ADN adducts. Approximately 5 and 10% of crops worldwide are spoiled by moulds and consequently must not be consumed by animals or humans. During the field growth and also post-harvest storage of pistachios (*Pistacia vera* L.), AF contamination produces health risks and economic losses [1]. Pistachios have a high risk of AF contamination [11,12] because of the open shell and premature pistachio opening that increase insect damage. In 2006, Iran detected that 37 % of their pistachio crops had an AFB_1 contamination of 5.9 ± 41.7 ng/g and 28% had an average total aflatoxin (AFt) level of 7.3 ± 53.2 ng/g [13]. In 2013, Iran had 23 % of their pistachio crop contaminated with AFB_1 (2.18 ± 13.1 ng/g) and 24 % with an average AFt level of 2.42 ± 14.7 ng/g [14]. In 2008, Morocco detected 45% contamination of their pistachios with AFB_1 (158 ± 6.3 ng/g) and 45% presented an average AFt level of 163 ± 5.4 ng/g [11]. In 2009, Tunisia detected a 53% pistachio contamination with AFt (21.8 ± 38.0 ng/g) [15]. There are also reports regarding AF contamination in foods from markets in Spain [16]. Mexico had a population of 112,336,538 in 2010 and a population density of 5920.5 inhabitants per km^2 with >18% of its population living in Mexico City, which includes the capital city or Federal District and the surrounding State of Mexico. Together, these both made a city of 20.4 million in 2012 [17], which is four times the population of Norway or Denmark and twice the

***Corresponding author:** Carvajal-Moreno M, Laboratorio de Micotoxinas. Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), 04510 México, DF. Tel: 5255-5622-9138; Fax: 5255-5550-1760; E-mail: magdac@ib.unam.mx

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population of Sweden. Mexico City is the third most populated city in the world after Tokyo and Delhi [18]. The Federal District is divided in 16 boroughs that receive food from all around the country, which makes it a reliable sampling place to obtain an idea of the pistachios consumed in the entire country. The population of the 16 boroughs in 2010 [19] is shown in Table 1. To date, there are no data regarding the pistachio consumption in Mexico and its comparison with foreign producer countries. The purpose of the research herein is to identify and quantify the AFs (B_1, B_2, G_1 and G_2) in pistachio seeds from Mexico City, and some samples from Istanbul (Turkey) and Santorini (Greece) using high performance liquid chromatography (HPLC). Despite aflatoxin contamination having been observed in several foodstuffs, the contamination of maize, peanuts, and oilseeds can be considered, in terms of diet exposure, the most important worldwide [20].

Materials and methods

Sampling

As a result of variable conditions that can occur during pre and postharvest, the aflatoxin contamination level among cereal grains and nuts such as peanuts, almonds, Brazil nuts, and pistachios within the same lot can have an extremely uneven distribution [21-24]. In a contaminated lot, just a few grains and nut kernels can have quite high concentration levels of aflatoxin, and most of them do not have detectable contamination [25].

The pistachios in shells of each market were placed in numerous saks of 20 kg each. Each sample was obtained taking pistachios with shell from different saks. The sample size of 100 g is accepted in the case of surveys, only bigger amounts are reccommended for international commerce burden [23]. In order to have a survey of the AF in pistachios of Mexico City, 100 g of samples of shelled natural pistachios were purchased from August 29 to September 13, 2012, from the three most important markets from each one of the 16 boroughs of Mexico City, which resulted in a total of 48 samples from 48 different markets. The pistachios were shelled and ground to powder and, in the case of Mexican pistachios, three subsamples (17 g), from each one of the three markets of the borough, were mixed to make a combined sample that represented the borough. Later each combined sample was blended (Black & Decker Mod. Crush Master, Mod. V2350BP) with methanol/water (80:20 v/v) for chemical analysis.

Sampling in Turkey and Greece: Six 100 g samples from different stores at the Spices Bazaar of Istanbul, Turkey were purchased on August 8th, 2010 and three samples, each of 100 g, from the market of Santorini, Greece were purchased on July 27th, 2010, and were analysed. All samples were frozen in Mexico upon arrival.

Validation of the method [26, 27]

Linearity (Calibration curves): The four AF calibration curves were obtained from separate one µg/mL stock solutions of AFB_1, AFB_2, AFG_1 and AFG_2 (Sigma-Aldrich, St. Louis, MO, USA). The AF standards were dissolved in one mL of benzene: acetonitrile (98:2, v/v) as suggested by the Official Method of Analyses AOAC 970.44 for aflatoxins [26-28] and then they were homogenised (Vortex 2 Genie Model G-560; 120 volts, 0.5 amperes, 60 hertz). Finally, one mL of each AF was diluted in HPLC grade MeOH and labelled.

The absorbance of the AF solutions in MeOH was measured at 362 nm in a spectrophotometer (Genesys, 10 UV Model, Thermo Electron Corporation, West Palm Beach, and FL33407 United States) to obtain the one µg/mL stock solution with different molecular weights and extinction coefficients [28].

Each AF standard had several dilutions (0.01, 0.05, 0.1, 0.5, 1, 2, 4, 5, 8, 10, 16, 20, 32, 40, 64, 70, 100, 128, 200, 600, 800, and 1000 ng/mL) that were dried in an oven at 40°C (Novatech BTC-9100). To derivatise the AF standard, 200 µL of ACN with 800 µL of derivatising solution were added. The derivatising solution had 5 mL of trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis MO, USA) with 2.5 mL of glacial acetic acid (Merck, Naucalpan, State of Mexico, Mexico) and 17.5 mL of deionised distilled water. The mixture, in an amber vial, was shaken for 30 seconds and then heated at 65°C for 10 min in a steam bath [29].

Average AF of Mexican 1-48 samples	Country	Borough/nhabitants in 2010	AF (ng/g) concentration mean rate					
			AFB_1	AFB_2	AFG_1	AFG_2	AFt	Average
1 -3	México	Álvaro Obregón/727,034	<LOD	0.03	0.07	1.07	1.17	0.29
4-6		Azcapotzalco/414,711	0.08	0.26	0.26	0.60	1.05	0.26
7-9		Benito Juárez/385,439	0.41	0.07	0.12	1.27	1.87	0.47
10-12		Coyoacán/620,416	<LOD	0.06	0.03	0.75	0.84	0.22
13-15		Cuajimalpa de Morelos/186,391	0.07	0.08	0.05	0.41	0.62	0.16
16-18		Cuauhtémoc/531,831	<LOD	0.09	0.03	0.79	1.25	0.23
19-21		Gustavo A. Madero/1,185,772	0.06	0.28	0.03	0.22	0.60	0.15
22-24		Iztacalco/384,326	<LOD	0.55	0.03	<LOD	0.58	0.14
25-27		Iztapalapa/1,815,786	0.17	0.16	0.12	<LOD	0.46	0.49
28-30		La Magdalena Contreras/239,086	0.25	0.05	0.06	0.67	1.03	0.26
31-33		Miguel Hidalgo/372,889	1.66	0.03	0.28	0.33	1.70	0.43
34-36		Milpa Alta/130,582	<LOD	0.03	0.04	0.17	0.24	0.06
37-39		Tláhuac/360,265	<LOD	0.04	0.04	0.27	0.35	0.09
40-43		Tlalpan/650,567	0.11	0.03	0.06	0.39	1.00	0.18
43-45		Venustiano Carranza/430,978	0.04	0.27	0.03	1.97	2.31	0.58
46-48		Xochimilco/415,007	1.56	0.51	0.38	0.85	4.26	1.08
Average AF of samples from:								
1-48	Mexico	48 markets, 3 from each one of 16 boroughs.	0.24	0.22	0.09	0.62	1.17	0.30
49-54	Turkey	Spices Bazaar	0.22	0.04	0.07	3.00	3.33	0.87
55-57	Greece	Santorini market	0.13	0.18	0.11	2.05	2.46	0.62

* One sample of 300 g divided in 3 subsamples of 100 g. <LOD are traces that count as zero.

Table 1: Aflatoxins in pistachio in Mexico City, Turkey and Greece.

Sixty µL were injected in the HPLC in triplicate. The equations for the calibration curves and the correlation coefficients (R^2) were obtained with Excel.

The limits of detection (LOD) were determined using the minimal concentration of the AF standard detected in the calibration curve. The limit of quantification (LOQ) was considered to be five times the value of the LOD [27].

Recovery rates: From AF standard, AFB_1, AFB_2, AFG_1, AFG_2, concentration of 1000 ng/mL, three concentrations (2, 10 and 100 ng/g) were independently prepared to spike five replications, of one gram of ground pistachio each, from a stock that had different basal AF concentrations. Basal contamination of the stock ground pistachio had 0.02 ng/g of AFB_1, 0.03 ng/g of AFB_2, 0.02 ng/g of AFG_1 and 0.32 ng/g of AFG_2. The five replications were done independently as suggested [28]. Each replication of one gram of ground pistachio was independently weighed and placed in 50 mL centrifuge tubes (Falcon). Each one gram of ground pistachio replications was spiked with the AF standard mentioned concentrations and three mL of MeOH, one gram of NaCl and 2 mL H_2O_d, were added and the solution was centrifuged at 4000 rpm (ALC 4235 with CWS freezing system) for 15 min. The supernatants were recovered, and then they were diluted in phosphate buffer (PBS) (1:4, v/v) pH adjusted to 7.4.

The diluted supernatant, equivalent to one gram of pistachio, was applied to a total aflatoxins (AFt) immunoafffinity column (Easi-Extract R-Biopharm Rhône LTD, UK) that was previously equilibrated with PBS (20 mL) at a speed of one drop per second.

The immunoafffinity column was washed with 20 mL of distilled water, and eluted with HPLC grade MeOH (1.5 mL) and distilled water (1.5 mL) by gravity into a labelled amber vial that was dried at 40ºC, derivatised and injected for HPLC analysis in triplicate (60 µL aliquots).

Selectivity: To confirm that the AFs had no interference with the pistachio matrix, a mixture of the four AF standards, dissolved in MeOH (3 mL) with one gram of NaCl and 2 mL of H_2O_d was measured alone and also applied to one ground pistachio samples (1 g), in a 50 mL centrifuge tube (Falcon) and centrifuged at 4000 rpm for 15 min. This procedure [26] was repeated to obtain a total of four replicates.

The supernatant, diluted in PBS (1:4, v/v) at pH 7.4, was applied to the immunoafffinity column in the same way as mentioned before [28]. The eluates were dried in an oven at 40ºC, derivatised and injected (60 µL aliquots) into the HPLC in triplicate.

Sample extraction method: Following known methods [30,31], 50 g of ground pistachio were blended with 100 mL of a methanol: water mixture (80:20, v/v) and 2 g of NaCl, and then filtered using fine pore paper. Then, the filtrate (2 mL) was diluted in 14 mL of PBS, and this mixture was applied to an equilibrated immunoafffinity column at slow flow rate, and washed with 20 mL of H_2O_d. The sample was eluted by gravity with 1.5 mL of HPLC grade MeOH followed by 1.5 mL of H_2O_d with reflux. The eluate (3 mL) was dried in an oven at 40ºC, and derivatised as mentioned previously [29].

Chromatographic conditions: The mobile phase was H_2O: ACN: MeOH (65:15:20, v/v/v) at a flow rate of one mL/ min. The fluorescence was detected at an excitation wavelength of 360-362 nm and emission of 425 nm for AFB_1 and AFB_2, and an emission wavelength of 450 nm for AFG_1 and AFG_2. ChemStation 32 was the program used for HPLC quantitation using a liquid chromatography (Series 1200) with an isocratic pump (G1310A Series DE62957044), fluorescence detector (G1321A Series DE 60456380) and autosampler (G1329A Series DE64761666), which were all from Agilent Technologies. The chromatographic column was a 4.6 × 250 mm Agilent Eclipse XDS-C18 with a 5 µm particle size.

AF sample quantification by HPLC: Sixty µL of derivatised eluate were applied in triplicate to quantify the AFs by HPLC. Utilising the areas and equations of each curve, the corresponding AF concentration was calculated (ng/mL) and corrected by the percentage of recovery as follows [28];

$$\text{Final corrected concentration} = \frac{100 \times \text{AF concentration in sample}}{\text{Recovery \% of AF}}$$

Statistical analyses

Kruskal-Wallis non-parametric statistical tests were used to analyse the data and compare the samples to check the relationship between concentration of AFs in the pistachios and their purchase sampling source. Later, a Wilcoxon range test was performed to determine the significant differences.

Results

Validation of the method

The analytical method fulfilled the validation criteria of the EC Regulatory Commission 2004/882.

Linearity (calibration curves): The correlation coefficient (R^2) and slope equation of each AF were: AFB_1 ($R^2 = 0.9973 \approx 1$, slope y = 2.8299 x); AFB_2 ($R^2 = 0.9908 \approx 1$, slope y = 1.7786 x); AFG_1 ($R^2 = 0.9969 \approx 1$, slope y = 1.7607x and AFG_2 ($R^2 = 0.9986 \approx 1$, slope y = 1.2411x). The retention times of the different validation parameters were compared to determine the accepted one for each AF: AFB_1 7.612-8.919 min, AFB_2 17.590-19.804 min, AFG_1 5.642-6.447 min, AFG_2 11.319-13.247 min.

Limit of detection (LOD) and limit of quantification (LOQ): The respective LOD and LOQ values were: AFB_1 (0.1 and 0.5 ng/g), AFB_2 (0.01 and 0.05 ng/g), AFG_1 (0.01 and 0.05 ng/g) and AFG_2 (0.5 and 2.5 ng/g).

Recovery percentage: The AF extraction method in pistachios was efficient, with a recovery percentage media of 88.06 % for AFB_1, of96.04 % for AFB_2, of 88.82 % for AFG_1 and 89.79 % for AFG_2.

Selectivity test : The selectivity test was the analytical method to determine whether the matrix (pistachio) interfered with the detection of the AFs. The four AF standards had no pistachio matrix interference. The proposed method has the power to discriminate between AFB_1, AFB_2, AFG_1 and AFG_2, and other pistachio components. The AF from the blank standards (BS) in comparison to the AF from the matrix (AFM) were: AFG_1 (BS 6.447 min and AFM 6.098 min); AFB_1 (BS 8.919 min and AFM 8.366 min); AFG_2 (BS 18.246 min and AFM 12.444 min) and AFB_2 (BS 19.804 min and AFM 18.490 min) which were in the same range of retention times and the peaks did not overlapped.

AF quantification in the pistachio samples

The results of the AFs in the pistachio samples are in Table 1. In general, all samples were contaminated with two or more AFs in low or trace concentrations. The tolerance levels for OMS [32] are 5µg/kg AFB_1 and10 µg/kg AFt and for FAO (2004) [33] are 20µg/kg AFB_1 and 35µg/kg AFt.

A total of 36.4% of the samples were contaminated with AFB_1 (0.10 to 4.15 ng/g), 100% with AFB_2 (0.01 to 4.00 ng/g) and AFG_1 (0.02 to

1.02 ng/g), 56.4 % with AFG_2 (0.46 to 8.09 ng/g), and 100% with AFt (0.04 to 8.39 ng/g). These concentrations are less than those reported in Iran [13], Morocco [11] and Tunisia [14].

In Mexico, the samples most contaminated with AFB_1 were from the Mixcoac market in the Benito Juárez borough (1.23 ng/g), the Tacubaya market in the Miguel Hidalgo borough (3.20 ng/g; 5.01 ng/g AFt) and the Xochimilco market and in the Xochimilco borough (4.15 ng/g AFB_1; 4.00 ng/g AFB_2; 1.02 ng/g AFG_1; and 7.04 ng/g AFt). AFB_2 contamination was 1.38 ng/g in the samples from the San Miguel market in the Ixtacalco borough. Moreover, increased AFG_2 (4.06 ng/g) was found in the Merced market, in the Venustiano Carranza borough with high AFt (4.43 ng/g). Sample 52 from the Spices Bazaar in Istanbul, Turkey had contamination with AFG_2 (8.09 ng/g) and an AFt value of 8.39 ng/g. The 3 samples from Greece had AFt 2.46 ng/g that increased from AFG_2 2.05 ng/g (Table 1).

Statistical analyses

The Kruskal-Wallis and the Wilcoxon range tests found statistically significant differences (<0.05) in the concentrations of AFB_1, AFB_2, AFG_1, AFG_2 and AFt of the pistachio samples and their origin, with the following AF (statistical values): AFB_1 (67.6629), AFB_2 (64.1586), AFG_1 (60.4021), AFG_2 (53.2218) and AFt (58.1913).

AFB_1 is the most mutagenic, teratogenic and carcinogenic compound of all AFs [9]. The Xochimilco borough samples (1.56 ng/g) are significantly different from the rest, except for those from the Benito Juárez borough (0.41 ng/g) (Figure 1a). For AFB_2 (Figure 1b), the Xochimilco samples (1.51 ng/g) were again different from the rest, except for those from Azcapotzalco (0.26 ng/g), Venustiano Carranza (0.27 ng/g), G.A. Madero (0.29 ng/g) and Iztacalco (0.54 ng/g). For AFG_1 (Figure 2a), the Xochimilco (0.38 ng/g) and Miguel Hidalgo (0.27 ng/g) boroughs were the most contaminated and different from the remaining ones, except the Azcapotzalco (0.10 ng/g), Benito Juárez (0.11 ng/g) and Iztapalapa (0.12 ng/g) boroughs as well as Santorini, Greece (0.11 ng/g). For AFG_2 (Figure 2b), the Santorini sample (2.05 ng/g) was the most contaminated and it was significantly different from the remaining ones, except the Cuauhtémoc (0.69 ng/g), Álvaro Obregón (0.71 ng/g), Benito Juárez (1.15 ng/g), Venustiano Carranza (1.52 ng/g) boroughs and Istanbul, Turkey (1.8 ng/g).

For AFt (Figure 3), the Xochimilco (4.08 ng/g) and Santorini (2.47 ng/g) boroughs were the most contaminated, with significant differences from the rest, except the Azcapotzalco (0.88 ng/g), Benito Juárez (1.75 ng/g), and Venustiano Carranza (1.84 ng/g) boroughs and Istanbul, Turkey (2.07 ng/g).

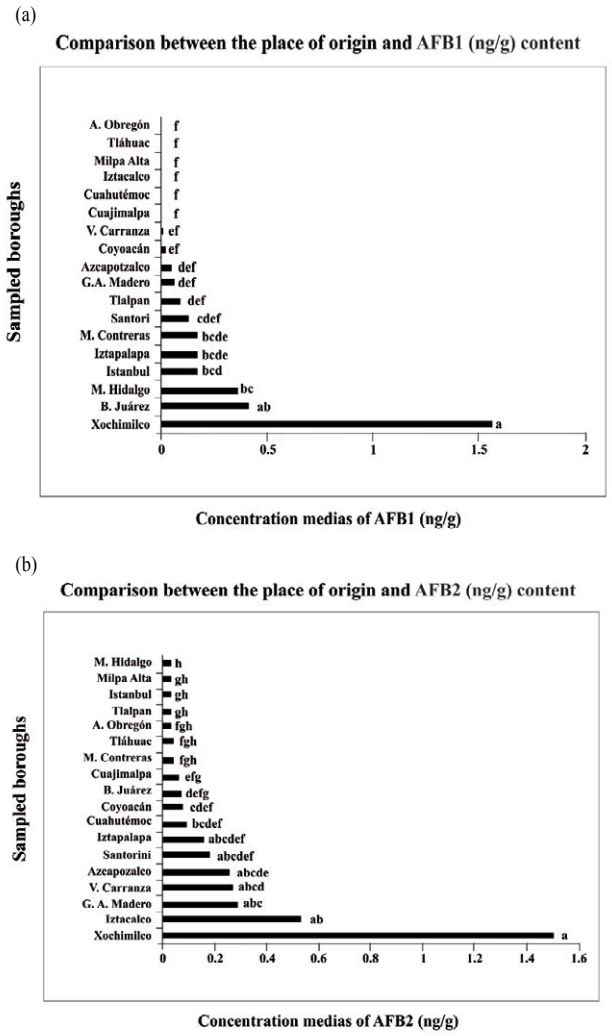

Figure 1: Wilcoxon/Kruskal-Wallis statistical test. Comparison between a) AFB1 concentration and purchase source, b) AFB2 concentrations and their purchase origin. Absence of common letters means a significant difference between samples.

Discussion

Pistachios are both a nutrient and a health risk. The present study helps humans to know the state of their pistachio, to prevent and avoid aflatoxins.A study [34] of *Aspergillus* molds in California pistachios, early split nuts had over 99% of the aflatoxin detected and navel-orangeworm-infected nuts had substantially more infection by several *Aspergillus* species, as well as over 84% of the aflatoxin detected. Also early splits with rough hulls had substantially more AF than early splits with smooth hulls [34]. AF levels were significantly lower in wounded kernels with hulls than in kernels of hulled pistachios. Both the seed coat and a water-soluble extract of hulls suppressed AF production by *A. flavus* [35].

The pistachio is an oilseed that is highly contaminated with AFs worldwide, and although the AF concentration in one gram of pistachio in Mexico is under the tolerance level to produce a mutation, which is 10 ng/g [36], the amounts consumed are much higher. Mold counts on nuts going into storage can be high [37], it is important that proper storage conditions (especially low relative humidity and absence of standing water) be maintained to avoid serious problems.

The *Aspergillus* mould attacks the pistachio both before harvest when the fruits are still in the tree and afterwards when the pistachios are in storage [38]. In addition, the early opening of the pistachio shell before harvest favours spore contamination by air or by insects. AF contamination is exacerbated by long-term storage with unhygienic conditions, such as high temperatures and increased humidity. The presence of AFB_1, AFB_2, AFG_1 and AFG_2 in the samples shows an invasion by *Aspergillus parasiticus* as well as *Aspergillus flavus,* the main producers [39], but no identification of the other species [40] was done.There was a higher contamination of the samples with AFG_2 than with AFB_1, which is the most mutagenic, teratogenic and carcinogenic of all AFs, in agreement with the results of other studies [9]. When *A. parasiticus* grows in a medium with a pH < 6.0, AF Group B synthesis is favoured, and with pH > 6.0, AF group G synthesis is stimulated

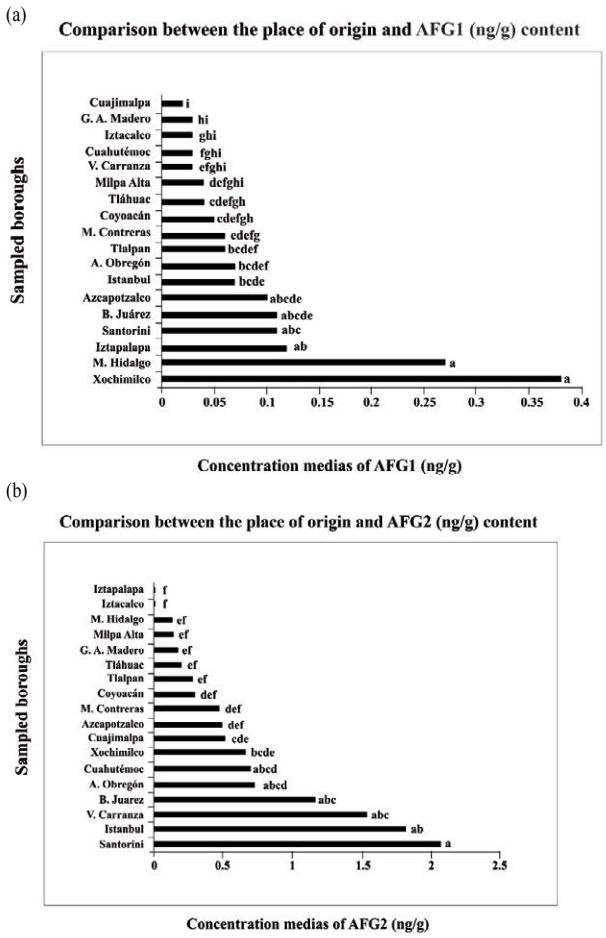

Figure 2: Wilcoxon /Kruskal-Wallis statistical test. Comparison between a) AFG1 concentration and their origin, b) AFG2 concentrations and their origin. Absence of common letters means a significant difference between samples.

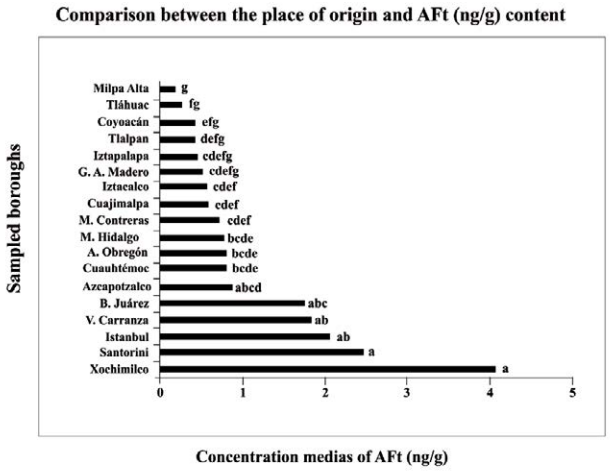

Figure 3: Wilcoxon /Kruskal-Wallis statistical test. Comparison of AFt concentration and their origin. Absence of common letters means a significant difference between samples.

[41]. In our case, it is possible that the pistachio pH was > 6.0, so after *A. parasiticus* invasion, the AF synthesis of group G, specifically AFG_2, occurred. All 57 pistachio samples (100%) from Mexico, Turkey and Greece were contaminated with AF; however, they were below the permitted levels given by OMS (5 µg/kg for AFB_1 and 10 µg/kg for AFt) and by FAO (20 µg/kg for AFB_1 and 35 µg/kg for AFt). A total of 36.4 % of the samples were contaminated with AFB_1 (0.10 to 4.15 ng/g), 100 % with AFB_2 (0.01 to 4.00 ng/g), 100 % with AFG_1 (0.02 to 1.02 ng/g), 56.4% with AFG_2 (0.46 to 8.09 ng/g) and 100 % with AFt (0.04 a 8.39 ng/g).The most contaminated samples were from the Xochimilco market in the Xochimilco borough for AFB_1 (4.15 ng/g) and AFG_1 (1.02 ng/g), from the Tulyehualco market in the Xochimilco borough for AFB_2 (4.00 ng/g), and from the Spices Bazaar market of Istanbul, Turkey for AFG_2 (8.09 ng/g) and AFt (8.39 ng/g).There was a statistically significant difference between the content of AF (B_1, B_2, G_1, G_2 and AFt) and the purchase origin of the samples. This oilseed is recognised as part of a healthy diet because they contain monounsaturated and polyunsaturated fats, which protect humans against heart diseases and reduce total cholesterol [42,43]. All pistachios samples are also a health risk, because most of them are contaminated with aflatoxins that are proven carcinogens. In the millions of years of co-existing together, it seems that the pistachio plants have developed high amounts of antioxidants as a reaction against the mould and its aflatoxins, such as caffeic acid that reduces the AFs by 99.5 %, quinic acid (90.2 %) and chlorogenic acid (88.5 %) [44]. Therefore, beneficial components, such as the antioxidants, and dangerous toxins appear together. The concentration of AFt found appears low, the homogenization was thorough and the analysed gram was representative, and a package of 100 g would have an average of 83 ng AFt /100g from Turkish pistachios or 30 ng AFt /100 g from Mexican pistachios. The metabolic activation of AFB_1 initiates the adduct formation that origins cancer. AFB_1 links to proteins such as albumin, ovalbumin, DNA and RNA, forming conjugates and adducts that can be detected in blood, urine and tissues of the organisms that ingested aflatoxins [45]. Adducts in tissues are the chronic exposition measure to aflatoxins and show the attack that DNA suffers in years of exposition. The presence of one adduct in 1, 000, 000 nucleotides is the measure of the malignicity of tumors in rat fed with AFB_1 [46]. As soon as a person gets old, his DNA accumulates more adducts capable of producing a mutation or the initiate a cancer [47].

Conclusion

In general, the amount of pistachios consumed as ingredient in desserts and ice creams is much higher than a gram, and all samples (100%) were contaminated with AF. Therefore, the consumption of this seed in years will produce a lot of stored adducts in DNA and this fact can be considered a risk factor for the development of cancer.

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