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Aflatoxins in Natural Peanuts (*Arachis hypogaea* L.) of Mexico: Validation of the Biochemical Methods for Extraction and Quantification

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Abstract

The peanut (*Arachis hypogaea* L.) is frequently contaminated with aflatoxins (AFs). Aflatoxins are toxic secondary metabolites – bifuran coumarins – produced by the fungi *Aspergillus* spp. AFs cause damage in animals and humans, including bleeding, vomiting, abortions, malformations, diarrhea and death. AFs can cause chronic liver damage and different cancers, immunosuppression, Reye's syndrome, kwashiorkor and marasmus. For the present research, 58 samples of peanut were obtained: 48 were purchased from the three major markets of the 16 boroughs of Mexico City, and eight samples from Turkey and two from India were used for comparison. The extraction and quantification methods for AFs in peanut were validated. AFs from 25 g peanut samples were extracted with 100 mL of methanol/water (80:20 v/v) with one gram of salt. The roles of sample origin and types of AF were compared, and their significance was obtained through statistical analysis using the non-parametric Wilcoxon/Kruskal-Wallis tests. The limits of detection of AF (ng g^{-1}) obtained from the calibration curves were AFB_1 (0.1), AFB_2 (0.01), AFG_1 (0.01) and AFG_2 (0.05). The results indicated that of the 58 samples, 80% were contaminated with AFB_1, and all of them had total aflatoxins (AFt). The statistical analysis revealed a significant difference for AFB_1, with the highest concentration of 44 µg kg^{-1} found in the Gustavo A. Madero Mexican borough. For AFB_2, the highest concentration was 80 µg kg^{-1} from the Benito Juárez Mexican borough. Only traces of AFG_1 were detected, with the highest of 0.64 µg kg^{-1} from the Tláhuac Mexican borough. Only traces of AFG_2 were present. All samples had an average AFt of 8.53 µg kg^{-1}.

Keywords: Aflatoxins; Peanuts; Biochemical contamination; Immunoaffinity columns

Introduction

Peanuts (*Arachis hypogaea*) are plants of the Leguminosae (Fabaceae) family that are sown and produced on 20 million hectares worldwide [1]. The global production of peanut was 39.67 million tons from 2015 to 2016 [2]. The largest peanut producer in the world is China, with 39% of the total production, followed by India, Nigeria and the United States of America [2]. In Mexico in 2014, peanut was sown on 59,414.83 Ha, with an average yearly production of 1.63 ton/Ha and a total production of 96,346.21 tons [3]. The total peanut consumption is approximately 160,000 tons a year, with 145,000 tons for human consumption, 6,000 tons for industrial processes, 8,000 tons for other uses, and 1,000 tons for sowing seed [3]. The peanut producer states in Mexico are Sinaloa (27,638.76 tons), Chihuahua (17,579.65 tons), Chiapas (12,015.63 tons), Oaxaca (10,263.83 tons), and Puebla (8,834 tons), accounting for 78% of the land dedicated to this crop [4,5] (Figure 1).

Peanuts have an underground fructification and an undetermined growth in warm tropical regions, making the yield very expensive and careful [3]. Peanuts are used to make candies, ice creams, cookies, pastry, beverages, sauces, cosmetics, shampoos, food supplements and antioxidants. Approximately 60% of the world's peanut production is consumed as oils [6]. Peanut seed has high nutritional and commercial values due to the presence fatty acids, carbohydrates, vitamins, calcium and phosphorus [7-9], and peanuts are the third most important source of plant protein, with fiber, arginine, and albumins representing 11% of the plant protein supply worldwide [10]. The oil content of peanuts is approximately 50-55%, from which 30% is linoleic acid and 45% is oleic acid. The latter can become rancid due to lipid oxidation, and the oleic/linoleic ratio increases the shelf life, which is used as a stability index for industrial applications [11].

During the growing period, many fungi damage the plant [7]. Aflatoxins (AFs) are toxic secondary metabolites–difurancoumarins produced mainly by the fungi *Aspergillus flavus* and *A. parasiticus* [12-14]. AF contamination is the main food security problem for peanuts in tropical and subtropical countries, where the temperature and humidity are high and favor the growth of *Aspergillus* spp. [15]. Peanut is a susceptible crop for the AF-producing fungi *Aspergillus flavus* and *A. parasiticus*, especially during dry seasons before harvest or during storage, when humidity and temperature control are inadequate [16-21]. AFs are toxic, low-molecular-weight metabolites that damage vertebrates, invertebrates, plants and microorganisms [22,23]. Peanuts are frequently contaminated with AFs that are produced mainly by *A. parasiticus* [12,13] before or after yield in natural and derived products such as milk and eggs [17-21], and in secretions and excretions of animals and humans fed with them [24].

AFs were discovered in 1960 when Brazil exported peanuts to the UK and 100,000 turkeys died of aflatoxicosis called "Turkey X Disease" [25-27]. AFs cause different types of damage [28-32] because they are mutagenic and carcinogenic. They can cause immunodeficiency,

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Figure 1: Peanut producer states of Mexico. Sinaloa, 25%; Chihuahua, 24%; Chiapas, 11%; Oaxaca and Puebla, 9%; and the remaining states, 22% (SIAP).

provoking thymic hypoplasia and thymocyte (immature leukocyte) depletion [33]. AFs are also teratogenic toxins that cause miscarriages and malformations [34]. After ingestion, these toxins are absorbed in the gastrointestinal tract and are biotransformed in the liver by the microsomal enzymes of cytochrome P450. The active form of AFB_1 (AFB_1 epoxide) affects DNA and RNA metabolism and protein synthesis [35]. AFs are hepatotoxic [36] and cause mainly hepatocarcinoma (HCC) [37] in individuals with hepatitis B virus (HBV) or those who are carriers of HBV antigen [38-41]. They are recognized grade I carcinogens, which has been proven for humans [42-44]. AFs are well-known and potent carcinogens depending on their dosage; the AF type; the time of exposure; the animal species; and the age, diet and nutritional state of the animal or human [45-50]. AFs are also related to other types of cancer [51,52], hepatitis, cirrhosis [53], Reye´s syndrome [54], kwashiorkor [55], hemorrhage, vomiting, diarrhea, and dwarfism [49]. High doses of AFs cause acute toxicity, sublethal doses produce chronic toxicity, and low levels over a long period produce cancer in many animals and humans [56].

The main AFs in peanuts are AFB_1, AFB_2, AFG_1 and AFG_2 [57]; of these, AFB_1 and AFG_1 are more frequent and occur in higher concentrations. The chemical structures and physicochemical properties of AFs have been described [27,58,59]. AFs show Blue (AFB_1 and AFB_2) or Green (AFG_1 and AFG_2) fluorescence [60], which is a useful property for their quantification [19,61-67].

The decreasing degree of toxicity is AFB_1>AFG_1>AFB_2>AFG_2, indicating that the terminal AFB_1 furane is the critical point that determines the AFs biological activity [67]. The increase in commerce and transportation of foods together with their fungi has forced countries to establish sanitary regulations to limit AF content in foods and agricultural products [68-70].

Soil is the main *A. flavus* and *A. parasiticus* contamination source for peanuts, as they develop underground, with their pods in contact with soil fungi [70].

The high concentration of AFs affects peanut quality worldwide, including discoloration, malodor, alteration of nutrients, and low

seed germination [71]. Poor-quality product cannot be consumed by humans, representing huge economic losses [72]. Peanut grains are a good substrate for mold growth when humidity and temperature are optimal for their development [73]. AF contamination can appear in all steps of the production cycle [50,74,75]. The minimal water activity to avoid toxigenic fungi growth is below 0.60 [76].

AF control measures for peanuts have been described [77-79]. Chemical control of AFs is expensive and not always efficient, but biological control reduces AF contamination before harvest by incorporating non-toxicogenic strains of the mold into the soil, where they compete with the toxicogenic strains for both infection sites and nutrients. These non-toxicogenic strains also protect the peanuts during storage [80].

The lethal dose (μg kg^{-1}) that kills 50% of the animals (DL_{50}) depends on the animal species and can range from 0.3 to 10 μg kg^{-1} [27]. For humans, it varies from 0.54 to 1.62 μg kg^{-1} [81].

It is important to determine the contribution of peanut to the inclusion of AF carcinogens in the human diet. The aim of the present study is to identify and quantify the AFs (AFB_1, AFB_2, AFG_1 and AFG_2) in 48 Mexican natural peanut samples from the three most representative markets of the 16 boroughs of Mexico City and from 10 samples from Istanbul, Turkey, and Bhopal, India, for comparison. The AF extraction was validated, and immunoaffinity columns and HPLC techniques were used for their identification and quantification.

Materials and Methods

Sampling

A representative sampling of the three main markets in each one of the 16 boroughs of Mexico City was conducted from August 30 to September 27, 2012, with 100 g of natural peanuts purchased per sample, which were then labeled and weighed. Random samplings (100 g each) of eight samples from an Istanbul market in Turkey and 2 samples from a Bhopal market in India were collected for comparison.

Chemical analysis

The seeds of 48 Mexican peanut samples, along with the eight samples from Turkey and the two from India, were chosen randomly and were individually analyzed. Twenty-five grams of each peanut sample was blended with 100 mL of methanol/water (MeOH:distilled $H_2O(H_2O_d)$ (80:20 v/v) (MeOH JT Baker) and 1 g of sodium chloride to obtain a homogeneous mass. This mixture was then centrifuged and decanted, and the supernatant was used in subsequent analyses.

Separately, total aflatoxin immunoaffinity columns (Easi-Extract Aflatoxin Biopharm, Rhône Ltd. Glasgow, Scotland, UK) that contained total AF (AFt) antibodies were adjusted to pH 7.4 using 20 mL of phosphate-buffered saline (PBS).

Four milliliters of the peanut supernatant equaling one gram was dissolved in 14 mL of PBS. These 16 mL mixtures were then applied into the immunoaffinity column. The AFs (antigens) were captured by AF antibodies. Next, the column was washed with 20 mL of distilled water to eliminate impurities. Finally, pure AFs were eluted with 1.5 mL of methanol HPLC purity, and another 1.5 mL of distilled water was used with reflux to separate the AFs from the agarose gel. The three mL of eluate was dried in an oven (Novatech BTC-9100 Houston, Texas, USA) at 40°C.

Derivatization

An AF derivatization was then performed for both to the AF standards (B_1, B_2, G_1, G_2) (Sigma-Aldrich, St. Louis MO, USA) and to the AFs eluted from the samples to increase the fluorescence of AFB_1 and AFG_1. The dried AFs were resuspended with 200 μL of acetonitrile (JT Baker, Xalostoc, Estado de México) and 800 μL of derivatizing solution, which consisted of 5 mL of trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO, USA), 2.5 mL of glacial acetic acid (Merck, Naucalpan, Edomex, México) and 17.5 mL of deionized water. The mixture was vortexed (Vortex G-560, Bohemia, NY, USA) for 30 seconds, and the vials were then submerged in a 65°C water bath for 10 minutes, as reported previously [82,83]. The samples were brought to room temperature, and 60 μL was injected by triplicate into the liquid chromatographer with a 20 μL loop for the quantification. The liquid chromatograph (Series 1200) had an isocratic pump (G1310A Serie DE62957044), a fluorescence detector (G1321A Serie DE60456380) and an autosampler (G1329A Serie DE64761666), all from Agilent Technologies. The chromatography column was an Agilent Eclipse XDS-C18, 4.6 × 250 mm, 5 μm particle size, and the HPLC software was ChemStation 32. The mobile phase was H_2O/ACN/MeOH (65:15:20 v/v/v). Vacuum filtration was used to degasify, and all solvents were HPLC purity grade and were purchased from JT Baker, USA.

Validation method

The high performance liquid chromatography (HPLC) method with a fluorescence detector was validated on respect to lineality (calibration curves), selectivity, limits of detection (LOD) and quantification (LOQ), and recovery percentage [84,85].

Linearity

The absorbance of each AF standard stock solution at one microgram per mL was measured in a spectrophotometer (Genesys 10 UV Thermo Electro Corporation UV/Vis), as reported previously [86]. The following formula was applied:

$$\frac{AF\ Absorbance \times molecular\ weight\ (MW)}{AF\ extinction\ coefficient} = 1 \mu g\ mL^{-1} concentration$$

The molecular formula, molecular weight (MW), fusion point, absorption at 360-362 nm, emission of fluorescence (425 nm for AFB_1 and 450 nm for AFG_1 and AFG_2) from the four AFs, and the extinction coefficients (ECs) were obtained from previous reports [58]. The MW and the EC were as follows: AFB_1 (MW=312; EC=21,800), AFB_2 (MW=314 and EC=24,000), AFG_1 (MW=328; EC=17,700) and AFG_2 (MW=330; EC=17,100).

Calibration curves

Calibration curves serve as the reference for the AF measurement in HPLC to obtain proportional results to the analyte concentration. Ten dilutions (0.1, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 ng mL^{-1}) were made from the 1 μg mL^{-1} standard stock solutions of AFB_1, AFB_2, AFG_1 and AFG_2. The calibration curves were constructed using the areas of the chromatographic peaks and the concentrations of the AF standards.

The AF standards were dissolved in 1 mL of a solution of benzene (Merck, Naucalpan, Edo. Mex., México): acetonitrile (98:2 v/v), as indicated [86], and a stock solution of 1 μg mL^{-1} was made. The calibration curves were then generated, and the linear regressions (R^2; $r \approx 1$) were obtained using the Excel program.

Selectivity

Selectivity is the degree by which the method can determine the retention time of the analyte without interference of the peanut matrix. This method analyzes 100 ng of each of the 4 AF standards alone and in the matrix. One gram of samples with less AFt from

the Gustavo A. Madero borough and the Río Blanco market were weighed and supplemented with 100 ng of each of the four AFs (AFB_1+AFB_2+AFG_1+AFG_2). Sixty microliters was injected for HPLC analysis, and the results were analyzed in triplicate.

Limits of detection (LOD) and quantification (LOQ)

LOD was calculated by a regression analysis of the AF calibration curve, it is the lowest amount that the equipment can measure. The LOQ is considered 5 times the LOD.

Recovery percentages

The recovery percentage is the efficiency by which the method can detect all of the analyte in a sample. Five Falcon 50 mL tubes with one gram of non-contaminated peanut (blank) were individually spiked with 100 μg mL^{-1} of each one of the four AFs and were compared with the mixtures of 100 μg mL^{-1} of the four AF standards. Each tube was supplemented with three 3 mL of MeOH, 2 mL of distilled water and one gram of NaCl, and the tubes were then centrifuged (ALC 4235 CWS) at 4000 rpm for 15 minutes. The supernatants were dissolved in PBS (1:4 v/v) and were applied to immunoaffinity columns, which were washed and eluted as explained in the section describing chemical AF extraction. The eluates were dried at 40°C in an oven and derivatized, and 60 μL was injected and quantified in triplicate via HPLC.

The recovery percentages were obtained by subtracting the AF of the matrix alone from the AF-spiked replicates, and the AF was then adjusted to 100% to calculate the precise amount of AF per sample.

AF quantification by HPLC

After injecting 60 μL of each sample in triplicate, the chromatographic peak areas and retention times of each sample were obtained (ng mL^{-1}) and were corrected with the recovery percentage to obtain the final concentrations.

Statistical analysis

The R statistical program [87], was used to perform the non-parametric Kruskal-Wallis test to determine the differences among AF contents in the peanut samples and the source boroughs. The Wilcoxon range test was applied to determine the significance of the differences.

Results and Discussion

Validation

The analysis of the data obtained for the different parameters revealed that the detection and quantification methods for the four AFs for the peanut samples from Mexico, Turkey and India fulfilled the desired requirements of reliability for the tests.

Linearity (Calibration curves)

Among the validation parameters of the aflatoxin method are the retention times (RT) that identify each AF, the AF (μg kg^{-1}) concentrations that quantify the AFs of each sample, the LOD and LOQ, the coefficient of determination (R^2) and the recovery percentage. The calibration curves were constructed using these data (Table 1). Recovery percentages for AFs were between 81.5 and 100%, indicating that the extraction method was reliable (Table 1).

Selectivity test

The blank or control chromatogram (Figure 2A) and the peanut matrix supplemented with the mixtures of the four AFs (Figure 2B) showed no interference between the AFs and the peanut matrix.

AFs	Retention times (min)	LOD	LOQ	R^2	Slope of Y line	Recovery %
AFB_1	7.612 to 8.16	0.1	0.5	0.9973	2.8299x	81.5
AFB_2	17.59 to 18.26	0.01	0.05	0.9935	1.7437x	100.0
AFG_1	5.64 to 5.93	0.01	0.05	0.9969	1.7607x	88.7
AFG_2	11.35 to 11.57	0.5	1.5	0.9986	1.2411x	97.0

AFs=Aflatoxins, LOD=Limit of detection, LOQ=Limit of quantification, R^2=Coefficient of determination, Y=slope, direction and steepness of the line, and the Recovery %=Recovery percentage.

Table 1: Validation parameters of the Aflatoxin method.

AF quantification in peanuts by HPLC

The AFt quantification in peanuts is shown in Table 2. From the 58 analyzed samples, 80% were contaminated with 3 out of the 4 AFs. AFB_1, the most important and toxic carcinogen [42,88], was present in 57% of the samples; 100% samples contained AFB_2 and AFG_1, but no samples were contaminated with AFG_2. All of the samples had AFt, with an average concentration of 8.53 μg kg^{-1}. Taking into account that the average Mexican consumes 1.8 kg of peanuts per year, we can conclude that the amount of AFs consumed based on peanut ingestion is 15.4 μg or 15,400 ng AFt per year [89].

Figure 3A-3E shows the separated AF concentrations in peanuts from Mexico City boroughs, with their statistical significances. The highest concentration calculated was 43.49 μg kg^{-1} AFB_1 from the Martin Carrera market, Gustavo A. Madero borough, followed by 24.73 μg kg^{-1} AFB_1 from the Tlacotal market, Iztacalco borough. The highest AFB_2 concentration was 79.52 μg kg^{-1} from the Portales market, Benito Juárez borough (Figure 3B). AFB_2 is not as carcinogenic as AFB_1, but it can be stored in animals and humans [81]. Only traces of AFG_1 were detected, with the highest being 0.64 μg kg^{-1} from the Tláhuac market and borough, but this AF was present in all of the samples (Figure 3C). No samples were contaminated with AFG_2; it is possible that the conditions for the synthesis of this AF (such as pH) were inadequate for its production [90].

From the foreign samples, the highest contamination had an average of 3.17 μg kg^{-1} AFt, and the samples from the Bhopal market in India had an average AFt of 0.73 μg kg^{-1}.

In Nigeria, approximately 64.2% of peanut samples were contaminated with AFB_1 (25.5 μg kg^{-1}) [91]. In Togo, 58.3% of the peanut samples had detectable levels of the fungus *A. flavus* [92]. AFs were detected in the hulls and peanut seeds in Brazil, where 20 samples (33.3%) were contaminated with AFB_1 (7.0 to 116 μg kg^{-1}) and 28.3% of the samples were contaminated with AFB_2 (3.3 to 45.5 μg kg^{-1}) [36]. There was more AF contamination in the peanut samples from Mexico than in those from Africa or Brazil.

The AF content in peanuts can be controlled; in the USA, AF concentrations should be <15 μg kg^{-1}, for sample quality to be approved [93]. The FDA establishes that peanuts must contain less than 20 μg kg^{-1} AFt [94].

The high amounts of AFs in natural peanuts can be due to the pre-harvest conditions, as the soil is the ideal place for *Aspergillus* growth [95]. The water activity (a_w) of the substrate plays a role in the growth of the fungus [71]. Dryness stress, high soil temperatures (>22°C) and physical damage during the growing stage of the sheaths can favor fungal invasion and AF production in peanuts [96-98].

AFB_1 was the most important AF; regarding source and concentration, Iztapalapa and Iztacalco were the most contaminated boroughs (Figure 3A). The most important commerce center for

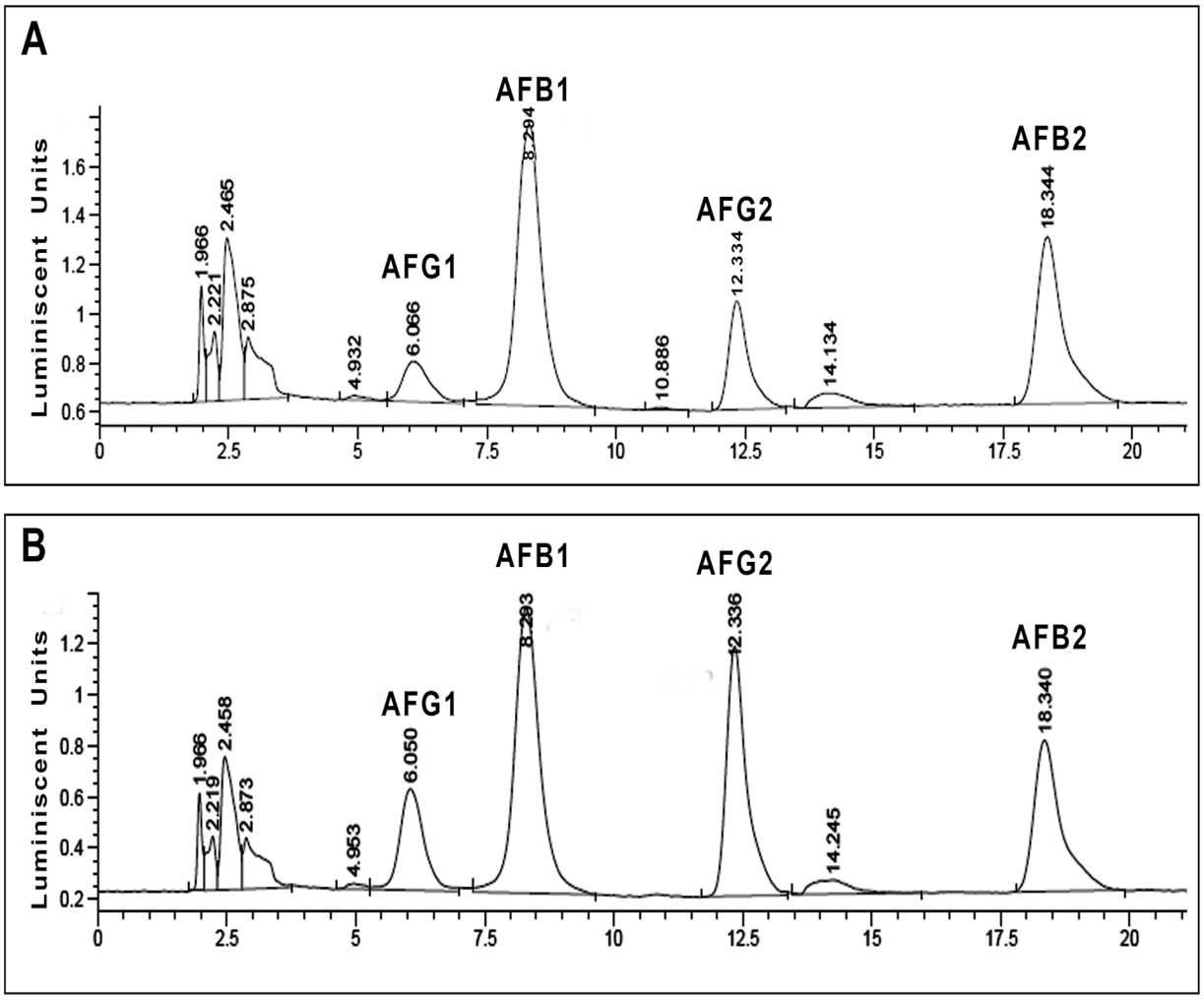

Figure 2: Selectivity test. A) AF standards, B) Peanut sample from the Río Blanco market in the Gustavo A. Madero borough, used as clean matrix, one gram with no AFs was supplemented with 100 ng of each of the four AFs (AFB_1+AFB_2+AFG_1+AFG_2), with no matrix interference. LU=Luminescent units given as a function of area.

peanuts is in Iztapalapa borough; therefore, it is extremely important that it was the most contaminated borough.

AFs have been detected in the cereals and foods of northern China and have resulted in lung and stomach cancers [99]. Intra-gastrointestinal administration of AFG_1 over a long-term period can produce adenocarcinoma in mouse lungs [100].

Natural peanuts commercialized in Mexico City are very susceptible to *A. parasiticus* and *A. flavus* and are slightly susceptible to *A. nomius* [101,102]. A. *parasiticus* produces the four AF types found in the samples, which is likely why AFt was high in the Benito Juárez, Iztacalco, Iztapalapa and Tláhuac boroughs (Figure 3D).

In the UK, AF legislation responded in agreement with the European Commission [103] for limits of 2 to 12 μg kg^{-1} of AFB_1 and 4 to 15 μg kg^{-1} of AFt. FAO and OMS adopted a level of 15 μg kg^{-1} AFt in natural peanuts [104]. In Mexico, the Official Mexican Norm NOM-188-SSA1-2002 establishes that 20 μg kg^{-1} AFt is the limit for cereals and foods (Figure 3D).

Statistical analysis

To determine whether there was a significant difference between the Mexico City boroughs and the foreign samples, a statistical study using the non-parametric Kruskal-Wallis test, was performed. This test determines whether two or more samples come from the same place [105].

The Kruskal-Wallis test yielded significant differences between the AFs and the sample places of origin, and there were also significant differences between the places of origin of each sample ($p<0.05$). The test suggested that each peanut is a different individual with a specific contamination depending on the crop conditions and that the AF distribution was random.

The statistical analysis compared AFs concentrations and places of origin that had similarities and differences by borough. The same letters show similar AF concentrations, therefore the samples from Iztacalco and Iztapalapa were not significantly different in terms of the AFB_1 concentration, although the samples from Iztacalco were more contaminated (Figure 3A).

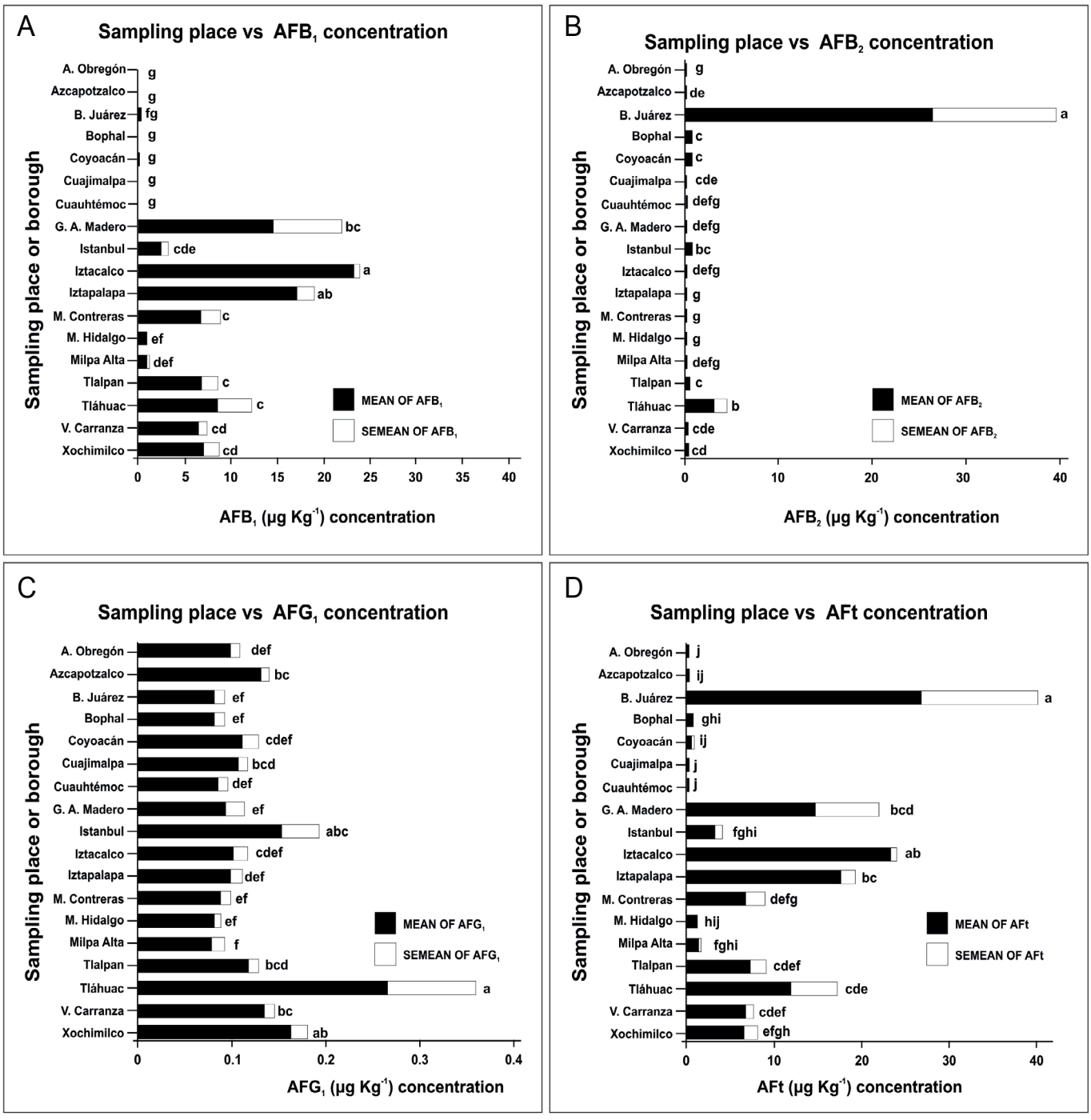

Figure 3: Statistical analysis and significance between peanut sample sources and AF concentrations. A=AFB_1, B=AFB_2, C=AFG_1 and D=AFt. Places label with the same letter (a, b, c, d, e, f, g, h, i, …) are not statistical different regarding concentration of the aflatoxin. SEMEAN=Standard error of means.

There was a significant difference in the AFB_2 concentration in the Benito Juárez borough compared with the other locations (Figure 3B).

The AFG_1 concentrations and the sampled locations are shown in Figure 3C. Most of the AFt contamination found in the samples was significantly different in terms of the place of origin, except for Coyoacán, Azcapotzalco and Cuajimalpa, Cuauhtémoc and Álvaro Obregón (Figure 3D).

Based on the Mexican AFt food regulations (20 µg kg^{-1}), seven markets surpassed the permitted limits, with concentrations of 21 to 80 µg kg^{-1}. The AF legislation of the European Commission tolerates a ≤ 2 µg kg^{-1} AFB_1 limit and a 10 µg kg^{-1} AFt limit. In this study, 13 samples exceeded the limitations by 10 to 80 µg kg^{-1}. In the study, 75% of the peanut samples had AFs, and several reports have indicated that all AFs are carcinogenic [106].

Source of samples	Market	Average AF (µg kg^{-1})				
		AFB_1	AFB_2	AFG_1	AFG_2	AFt
Álvaro Obregón §	Melchor Múzquiz	<LOD	0.04	0.09	<LOD	0.13
	Olivar del Conde	<LOD	0.04	0.09	<LOD	0.14
	Corpus Christi	<LOD	0.04	0.11	<LOD	0.15
Azcapotzalco §	Azcapotzalco	<LOD	0.10	0.15	<LOD	0.25
	Prohogar	<LOD	0.17	0.12	<LOD	0.28
	Nueva Santa María	<LOD	0.11	0.12	<LOD	0.23
Benito Juárez §	De Mixcoac	<LOD	0.09	0.07	<LOD	0.16
	Portales	0.56	79.04	0.09	<LOD	**79.69***
	Independencia	<LOD	0.06	0.09	<LOD	0.15
Coyoacán §	Ajusco-Moctezuma	0.13	1.12	0.09	<LOD	1.39
	Santo Domingo	<LOD	0.06	0.14	<LOD	0.15
	De Coyoacán	<LOD	0.09	0.10	<LOD	0.19
Cuajimalpa §	Contadero	<LOD	0.14	0.13	<LOD	0.26
	Rosas Torres	<LOD	0.15	0.09	<LOD	0.24
	Cuajimalpa	<LOD	0.12	0.10	<LOD	0.22
Cuauhtémoc §	Hidalgo	<LOD	0.10	0.11	<LOD	0.21
	Arcos de Belén	<LOD	0.12	0.07	<LOD	0.19
	San Juan	<LOD	0.06	0.08	<LOD	0.13
Gustavo A. Madero §	Río Blanco	<LOD	0.06	0.05	<LOD	0.11
	De la Estrella	<LOD	0.06	0.06	<LOD	0.12
	Martín Carrera	43.39	0.16	0.17	<LOD	**43.71***
Iztacalco §	San Miguel Iztacalco	23.94	0.11	0.07	<LOD	**24.12***
	Tlacotal	24.73	0.18	0.13	<LOD	**25.04***
	Santa Anita	20.92	0.10	0.09	<LOD	**21.11***
Iztapalapa §	Central de Abastos	11.04	0.07	0.09	<LOD	**11.20***
	Santa Cruz Meyehualco	19.48	0.09	0.12	<LOD	**19.68***
	Jacarandas	21.38	0.08	0.09	<LOD	**21.55***
La Magdalena Contreras §	Cerro del Judío	14.69	0.08	0.09	<LOD	**14.86***
	La Loma	4.94	0.06	0.09	<LOD	5.09
	Turistico La Magdalena	0.13	0.06	0.09	<LOD	0.19
Miguel Hidalgo §	Tacubaya	1.24	0.04	0.08	<LOD	1.36
	Plutarco Elías Calles	0.52	0.04	0.09	<LOD	0.65
	Argentina	0.85	0.04	0.07	<LOD	0.96
Milpa Alta §	San Antonio Tecómitl	0.64	0.10	0.07	<LOD	0.91
	Villa Milpa Alta	1.03	0.06	0.06	<LOD	1.15
	San Pedro Actopan	1.76	0.16	0.11	<LOD	2.03

Tláhuac §	Colonia del Mar	3.81	0.22	0.09	<LOD	1.59
	Mercado de la Nopalera	1.16	0.09	0.06	<LOD	1.31
	Mercado de Tláhuac	23.30	8.77	0.64	<LOD	**32.71***
Tlalpan §	Tlalcoligia	0.43	0.12	0.09	<LOD	0.64
	Torres de Padierna	12.45	0.64	0.12	<LOD	**13.21***
	De la Luz	7.50	0.16	0.14	<LOD	7.80
Venustiano Carranza §	La Merced	8.24	0.12	0.14	<LOD	8.49
	Jamaica	8.12	0.45	0.15	<LOD	8.72
	San Ciprian	2.48	0.16	0.12	<LOD	2.76
Xochimilco §	De Xochimilco	6.17	0.11	0.12	<LOD	6.40
	San Gregorio Atlapulco	11.10	0.56	0.19	<LOD	**11.85***
	Tulyehualco	0.92	0.12	0.17	<LOD	1.22
India	Bophal market	<LOD	0.54	0.09	<LOD	0.72
		<LOD	0.64	0.08	<LOD	0.73
Turkey	Istanbul Spices market	8.76	0.45	0.50	<LOD	9.70
		8.70	0.15	0.15	<LOD	9.30
		0.31	0.58	0.23	<LOD	1.01
		0.43	0.41	0.05	<LOD	0.90
		0.44	0.62	0.07	<LOD	1.14
		0.13	0.93	0.06	<LOD	1.03
		<LOD	1.23	0.10	<LOD	1.32
		<LOD	0.82	0.07	<LOD	0.89

§=Mexican borough; *=AFt (μg kg^{-1}) above the European Commission tolerance limit.

Table 2: Aflatoxin concentration (μg Kg^{-1}) in peanut.

Most of the analyzed samples had traces of AFt, but the boroughs Benito Juárez (80 μg kg^{-1} AFt), Gustavo A. Madero (44 μg kg^{-1} AFt), Iztacalco (25 μg kg^{-1} AFt), Iztapalapa (22 μg kg^{-1} AFt) and Tlalpan (33 μg kg^{-1} AFt) had values that surpassed the AF tolerated limits established by the Mexican Official Norm (Norma Oficial Mexicana) NOM-188-SSA1-2002. This fact illustrates the great health risk problem for Mexicans due to their high peanut consumption.

The Commission Regulation of the European Union (2010) has established tolerance limits of 5.0 ng g^{-1} for AFB_1 and 10.0 ng g^{-1} for AFt in spices and 2.0 ng g^{-1} for AFB_1 and 4 ng g^{-1} for AFt in peanuts and other seeds, such as oilseeds and industrialized food products for human consumption. The Commission also established limits of 5.0 ng g^{-1} for AFB_1 and 10.0 ng g^{-1} for AFt for dry fruits [107].

There have been many reports regarding AFt contamination in peanuts, nuts, dry fruits, spices, rice, maize, and soybean [92,108-112].

Some substances, such as oleic and linoleic acids in maize, might control the mutagenicity of AFs [113,114]. In our case, it seems that the peanut and the fungus *Aspergillus* living together for millions of years resulted in the plant developing protections against AFs. As an example, African groundnuts produce three stilbene phytoalexins that are cis and trans isomers closely related to 3,5,4'-trihydroxy-4-isopentenylstilbene (4-isopentenylresveratrol) [115]. Resveratrol acts against fungal spore germination and hyphal extension, so although AFs are present, they are less harmful for the groundnut [116].

Peanuts produce resveratrol, a polyphenolic phytoalexin, in response to external stimulus, such as fungal damage [117]. Resveratrol can be found in wines [118], legumes [119], berries [120] and pistachios [121]. The presence of AFs in groundnuts is frequent, along with the resveratrol produced as a protection, demonstrating the characteristics of a dialectic relationship in the union of opposites in the peanut, a carcinogen and an anticarcinogen together. This example illustrates the fight for survival between the peanut and the fungus with its toxins, with results that affect humans indirectly as consumers of both. AFs are recognized mutagens, but when they are extracted directly from maize or rice, they are not detected as mutagenic in the Ames test [113,114,122]. However, as soon as matrixes are treated with AF standards, their mutagenicity becomes obvious. It is highly likely that this phenomenon also occurs in peanut, which would indicate that while the peanut plant cannot avoid the fungus, with the formation of the antioxidant resveratrol, the plant can combat the AF's mutagenicity. The problem with this relationship is that when peanut seeds and AFs are ingested and digested by humans, everything is dissociated; in the liver, every food component is broken down, and the AFB_1 is then free to be mutagenic when it links to DNA over time; however, this fact remains to be properly demonstrated.

The interest for resveratrol, as a protector against cancer, lies in its capacity to suppress the proliferation of a wide variety of cancer tumor cells, including lymphoid, multiple myeloma, breast, prostate, stomach, colon, pancreas, thyroid, melanoma, head and neck squamous cell carcinoma, ovarian and cervical carcinomas [123]. In heart coronary diseases, the peanut has medicinal properties, as it can help to prevent heart disease by lowering cholesterol; it also stimulates and regulates digestion [124]. In addition to these beneficial effects, resveratrol has demonstrated neuroprotective effects against β-amyloid-induced neurotoxicity in rat hippocampal neurons with the involvement of protein kinase C and acts as an anti-inflammatory compound [125]. Stilbene blocks the multistep process of carcinogenesis at various stages: tumor initiation, promotion, and progression. One of the possible mechanisms for its biological activities involves downregulation of the inflammatory response through inhibition of the synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis and inhibition of activated immune cells. Resveratrol has an effect on the lifespans of yeast and flies and has potential as an anti-aging agent in treating age-related human diseases [126]. Due to these beneficial properties, resveratrol is used in food complements to obtain functional foods. The changes in trans-resveratrol caused by ultraviolet light and the determination of trans-and ci s-resveratrol contents in Spanish white wines have been reported [127,128].

This sampling was carefully performed using validated methodology and included all 16 boroughs that form Mexico City, which is a peanut

gathering and storage center and receives peanuts from all over the country. This research shows that it is necessary to review the AF levels in peanuts, as they can be very high in some samples. AFs accumulate in DNA, and peanut consumption is continuous; therefore, the ingestion of this oilseed can be a risk for human health.

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