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Presence of Aflatoxins (Mutagens and Carcinogens) in Industrialized Chili Sauces

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Abstract

Aflatoxins (AFs) are the most frequent mutagens, teratogens and carcinogens detected in food, and they are also present in all types of chili peppers and sauces made with them. Fifty-two different industrialized chili pepper sauces from markets in Mexico were analyzed for the presence of aflatoxins (AFB1, AFB2, AFG1 and AFG2). The chemical AF extraction and high performance liquid chromatography methods were validated based on the following parameters: selectivity, linearity (calibration curves), recovery percentages, limits of detection (LOD) and quantification (LOQ). The square of correlation coefficients (R2) were AFB1: 0.9973; AFB2: 0.9892; AFG: 0.9969; and AFG2: 0.9986. All curves were correct, with an average regression coefficient of R2 > 0.9892. The percentage of recovery, was 83% for AFB1 , 75% for AFB2, 96% for AFG1, and 81% for AFG2. The Limits of Detection (LOD) were 0.1 ng for AFB1, 0.01 ng for AFB2, 0.01 ng for AFG1, and 0.5 ng for AFG2.

For the statistical analysis, the different chili sauce groups were analyzed and compared using a Kruskal-Wallis test, and the results showed no statistically significant differences among the samples with respect to the total aflatoxins (AFt) amounts. The removal of all high mutagenic levels of AFG1 (> 10 ppb) from the analysis did not change the results. Eight chili pepper sauces (15%) from the different groups surpassed the 10 ppb AFt tolerance limit of the Codex Alimentarius and had 15 to 116 µg kg-1 AFt. The average AFt from the 52 samples was 3.69 µg kg-1, which were all within the globally accepted limits. Thus, these results suggest that there is no consistent basis for warning the public about AFs in the most commonly used salsas, but their use should be moderated because many are not safe to eat.

Keywords: Aflatoxins; Food carcinogens; Chili pepper sauces; Spices

Introduction

Aflatoxins (AFs), or bis-dihydrofuran-coumarins, are biologically active secondary metabolites primarily produced from the fungi Aspergillus flavus, A. parasiticus and A. nomius. These toxins have been reported as the most dangerous food mycotoxins, with teratogenic, mutagenic, immunotoxic, and carcinogenic effects, and AFs are considered Grade I carcinogens for humans, which act in concentrations of micrograms per kilogram [1,2].

The major AFs detected in chilies are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). AFB1 is considered the most common and dangerous aflatoxin, as this compound is easily absorbed and accumulates in the liver.

AFs are also considered the most important mycotoxins because they are the most frequently detected in many types of foods, including cereals [3], oilseeds [4], spices [5], milk [6,7] and dairy products, eggs [8], and animal derivatives, such as poultry litter [9] or hen breast muscle [10]. AFs are also biomarkers associated with DNA, RNA and proteins such as albumin, can accumulate in nucleic acids for years as adducts, and are active carcinogens [11]. Chili peppers are spices particularly susceptible to Aflatoxin (AF) contamination [5].

The preparation of sauces diminishes the amount of AFs compared with raw chili peppers, but chili pepper sauces also contain these mutagenic toxins [12]. A total of 170 samples of chili peppers obtained from an open market in Pakistan showed AFs in 39% of chili sauce, 59% of crushed chili peppers and 56% of chili powder samples compared with 29, 54 and 58% of samples, respectively, obtained from food restaurants that might have more time heating or other ingredients [12]. Another study found AFs in 12, 27 and 35% of chili sauce, crushed chili and powdered chili samples, respectively, obtained from an open market were higher than the European Union (EU) limit for total AFs, compared with 8, 32, and 41% of samples obtained from food restaurants [13]. Another study in Pakistan reported that the daily intake of AFs was 3.3 µg kg-1 [12].

The consumption preferences of regional peppers in 18 towns in the Central Valley of Oaxaca, Mexico was surveyed in 1287 people over 15 years of age [14]. The results showed that individuals preferentially consumed 'Jalapeño', 'Chile de Árbol', 'Chile de Agua', 'Chile Serrano', 'Solterito' and 'Tusta' hot peppers on the basis of their flavor and pungency. The regional peppers were found to be consumed by families in sauces, as fillings, as whole roasted vegetables or in raw slices and ranged from a few pods to one kilogram per week. The consumption preferences were found to be cognitive-affective based on stimulus perception, and these preferences transformed into needs within a socio-cultural and territorial context [14].

Previous studies have concluded that the high amounts of AFs contaminating chili pepper samples consumed by populations from Bolivia and Peru are associated with high gallbladder cancer incidence rates [15]. By far, red chili peppers showed the highest AF-positive percentages in 603 food samples from Catalonia, Spain [16].

A survey of 121 samples of ethnic foods, spices and sauces was conducted in the UK to determine the levels of mycotoxins. AFs were detected in chili oil and chili paste. The highest mycotoxin levels and occurrence frequencies were observed for chili powder, curry powder and ginger [17].

AF contamination of ground red pepper sold in Turkey was found to be higher than the total aflatoxin (AFT) and AFB1 legal limits, while one packed sample was 89.99 ppb over the legal limit for AFB1. Further, mold and yeast counts in unpacked ground red pepper were detected over the legal limits in 5.9% (5/85) of the samples [18].

In another study aflatoxin levels were slightly affected by radiation doses of 2 and 4 kGy and showed a nonsignificant reduction of 6% at the highest radiation dose of 6 kGy. The different effects of gamma radiation on molds and AFs, which were only slightly affected, can be explained by the target theory of food irradiation, which states that the likelihood of a molecule being inactivated by gamma rays increases with increasing molecule size [19].

In an overview of systematic reviews on the adverse effects of herbal medicines, Capsicum spp. had only minor adverse effects (burning sensation and pain) and was found to be reasonably safe [20]. However, previous studies have reported abdominal adenocarcinoma in mice fed 100 mg of red chilies per day for 12 months; no tumors were observed in the control animals. Neoplastic changes in the liver and intestinal tumors were observed in rats fed red chili powder at 80 mg/kg day-1 for 30 days, and intestinal and colon tumors were observed in rats fed red chili powder and 1,2-dimethyl hydrazine; no tumors were observed in the controls. In acute oral toxicity studies, capsaicin induced gastric fundal hemorrhage in some of the animals that died [21].

AFB1 (10-120 μg kg-1) was detected in 18 of 125 samples of spices, and red pepper showed the highest incidence [22].

An assessment of the total aflatoxin levels in 36 unpacked powdered red pepper samples obtained from Istanbul revealed that 32 samples (88%) contained AFt ranging from 0.2-106.4 μg kg-1, and 16 samples (44.4%) showed AFt levels above the regulatory limit of 10 μg kg-1 for total AFt in Turkey. Thus, more precautions concerning the production, transport, harvest, and storage of red pepper should be taken to prevent the toxic and carcinogenic effects of AFt [2].

Hot peppers packed in jute bags in Pakistan and stored at 25 °C for 150 days showed 75% higher AF contamination (1.50 μg kg-1) compared with those packed in polyethylene bags. A gradual increase in temperature during the prolonged storage of hot peppers in combination with aeration might contribute to the increase in fungal biomass and Aspergillus proliferation with subsequent aflatoxin production [23]. The U.S. Food and Drug Administration recognize Capsicum and paprika as safe for use in food, although AFs have been detected as contaminants. The AF contents of 130 commercial spice preparations, including chili pepper, were analyzed. The samples were obtained from various supermarkets, shops and market stalls in Ireland. AFB1 showed the highest incidence of contamination in spice preparations and was detected in 20/130 samples. The highest concentration of AF (27.5 μg kg-1), was detected in a sample of chili powder [24].

Thus, chili sauces are an important foodstuff for further analysis, and the aim of the present study was to validate the analytical method for AF detection and to quantify the health risk posed by AFs in the hot chili sauces in Mexico.

Materials and Methods

Sampling

A total 52 different samples of hot chili pepper sauces were obtained from supermarkets in Mexico City. The sample size was 40 g of chili pepper sauce, and the samples were dried in an oven (Novatech BTC-9100, Houston, Texas, USA) and weighed on a balance (Ohaus 700 Series, Parsippany, NJ, USA). Each sample was labeled and homogenized in a blender (Oster Mod. 465-43, Chicago, USA) to generate a representative AF concentration.

AF extraction and purification: Each sample of 40 mL of hot sauce was diluted with 120 mL of acetonitrile/ waterdist (80/20 v/v) (JT Baker, Center Valley, PA, USA) solution, and 2 g of NaCl was added. Subsequently, each sample was individually homogenized for 1 minute and filtered through Whatman N° 4 filter paper.

Three milliliters of the filtered solution, equivalent to one gram, was diluted in 14 mL of phosphate-buffered saline (PBS): 10.0 g KCl, 10.0 g KH2PO4, 58.0 g of Na2HPO4, 400 g of NaCl, and 2.5 g of NaN3 (JT Baker). The buffer volume was adjusted to 5 L with waterdist, and the pH was adjusted to 7.4 [25].

The immunoaffinity column for AFt (Easi-Extract R-Biopharm Rhône LTD, UK) was equilibrated with 20 mL of PBS and subsequently loaded with 16 mL of diluted filtrate, equivalent to one gram, at a speed of one drop per second. The column was washed with 40 mL of waterdist and air was subsequently passed through it. The AFs from the sample were eluted and separated from their antibodies in the agarose gel using 1.5 mL of high performance liquid chromatography (HPLC)-grade MeOH, by gravity and reflux with 1.5 mL of waterdist. A total of 3 mL of eluate was collected in a labeled amber vial. The eluates were then dried in a 40 °C oven.

Derivatization: For the derivatization of the AF standards for the calibration curves, 200 μL of acetonitrile (ACN) and 800 μL of derivatizing solution (made up of 5 mL of trifluoroacetic acid (ATF) (Sigma-Aldrich, St. Louis MO, USA), 2.5 mL of glacial acetic acid (Merck, Naucalpan, Edo. Mex., México) and 17.5 mL of deionized water) were added to the reactive compounds, followed by homogenization in a vortex (Vortex G-560, Bohemia, NY USA) for 30 seconds. The vials were subsequently placed in a 65 °C water bath for 10 min. The dried samples were dissolved in 100 μL of ACN and 400 μL of derivatizing solution to increase the fluorescence, as previously reported [26,27]. Finally, 200 μL of the derivatized sample was injected into HPLC inserts to quantify the AFs.

AF quantification and identification by HPLC: The AF standards and derivatized samples were warmed to room temperature and, from the 200 μL in the vial inserts; 60 μL of the sample was injected into the HPLC system with a 20-μL loop for analysis in triplicate. The HPLC (Series 1200) was equipped with an isocratic pump (G1310A Series DE62957044), a fluorescence detector (G1321A Series DE60456380) and an autosampler (G1329A Series DE64761666) (all from Agilent Technologies), a chromatographic C18 column from Agilent Eclipse

(XDS-C18, 4.6 × 250 mm, 5 µm particle size), and ChemStation 32 software. The mobile phase or isocratic solution for HPLC contained H2O/ACN/MeOH (65:15:20 v/v/v), filtered under a vacuum for degasification. The following solvents were used: methanol (CH3OH) (JT Baker, USA), acetonitrile (CH3CN) (JT Baker, USA) and distilled water (H2Odist). All solvents were of HPLC grade purity.

Validation of the method

Validation provides certainty that the chemical method was properly used and involves measuring the following parameters [28]:

1. Selectivity, 2. Linearity (calibration curves), 3. Recovery percentage, and 4. Limits of detection (LOD) and quantification (LOQ).

Selectivity: Selectivity is the parameter that determines whether the analyte (100 ng of the four AFs) and the matrix, homemade Northern Buffalo sauce, with no AF contamination, interfere with each other. One gram of the matrix without AF contamination was weighed and enriched with a combination of 100 ng of each of the AFs (AFB1 + AFB2 + AFG1 + AFG2). These samples were analyzed using HPLC.

Linearity: Linearity is the ability to obtain results that are in proportion to the analyte concentration. An individual stock solution of one µg mL-1 of each AF (AFB1, AFB2, AFG1 and AFG2), determined through spectrometry, was prepared as previously described [29]. The calibration curves were constructed after plotting the peak areas against the concentrations of each AF standard solution. The AF standards were prepared in 1 mL of benzene/ACN (980:20 v/v) and subsequently homogenized [29]. The absorbance of the AF standards was measured at 362 nm using a spectrophotometer (Genesys 10 UV Model Thermo Electron Corp., Massachusetts, USA). Additionally, the molecular weight (MW) and the extinction coefficient (CE) were calculated [29]. The following molecular weights (MW) and extinction coefficients (EC) were calculated for each AF standard solution: AFB1 (MW 312, EC 21,800), AFB2 (MW 314, EC 24,000), AFG1 (MW 328, EC 17,700), and AFG2 (MW 330, EC 17,100).

The calibration curves included the following concentrations of the standards: AFB1 (0.1, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 ng mL-1), AFB2 (0.01, 0.05, 1, 5, 10, 20, 40, 70, 100 and 200 ng mL-1), AFG1 (0.01, 0.05, 0.1, 0.5, 1, 4, 16, 100 and 128 ng mL-1) and AFG2 (0.5, 1, 2, 4, 8, 16, 32, 64, 100, 200, 600, 800 and 1000 ng mL-1). The linear regression for each AF was obtained using Microsoft Excel.

Recovery percentage: The recovery percentage determines the efficiency of the method for detecting all of the studied analytes in a sample. One gram of a chili pepper sauce type with no AF contamination was placed in a 50-mL centrifuge tube (Falcon) and fortified with 100 ng g-1 of each of the 4 AFs. Three mLs of MeOH, 2 mL waterdist and 1 g NaCl were added to each sample, and the samples were subsequently centrifuged (ALC 4235 with cooling system CWS) at 4000 rpm for 15 minutes. The supernatant was diluted (1:4 v/v) with PBS at pH 7.4, and applied to an immunoaffinity column that had previously been equilibrated with 20 mL of PBS. The immunoaffinity columns were washed with water and eluted with 1.5 mL of HPLC-grade MeOH, followed by reflux with 1.5 mL of water to break the agarose gel and recover the AFs.

The eluates were dried in a 40 °C oven, followed by derivatization, and 60 µL of these samples was injected into the HPLC in triplicate. The amount of each recovered AF percentage was adjusted to each AF concentration in the samples.

Limits of detection (LOD) and quantification (LOQ): The limit of detection (LOD) is the smallest reliable quantity of AF detected using HPLC and reproduced in the matrix. The LOD was calculated using a regression analysis of the calibration curve for each AF. The limit of quantification (LOQ) was calculated as 5 times the LOD.

Statistical Methods

A Kruskal-Wallis test, which uses ranges of sampling data from three or more independent groups, was applied to the data. This test is used to confirm the null hypothesis that the independent samples were derived from distributions with the same medians. The alternative hypothesis is that groups have different medians, and therefore different distributions. It is an alternative to analysis of variance (ANOVA) when one or more of the assumptions are not met. In this particular case the assumption of Normal distribution is not realistic, in fact the data are not symmetrical and the use of non parametric test are recommended. For the statistical analysis, the different chili sauces were grouped into 8 similar chili sauce types based on their ingredients: 1) Smoked "Chipotle", "Meco" and "Pasilla" chilies, 2) Fresh "Jalapeño" peppers 3) Fresh "Serrano" peppers, 4) Dry "Morita", 5) Pungent "Habanero", 6) Aged pungent red peppers "árbol", "guajillo" and "piquín", 7)"Chamoy" and 8) Different or not determined types of chili peppers.

Results and Discussion

Validation of the method

Selectivity: The selectivity chromatograms for the chili pepper sauce matrix were blank and fortified with 100 ng g-1 AFB1, AFB2, AFG1 and AFG2, respectively. There was no interference between the retention times of the different AFs and the sauce matrix; therefore, the separation was correct.

Linearity: The concentrations for each AF curve produced the following linear equations and square of correlation coefficients (R2): AFB1: y= 2.8299 x and R2 = 0.9973; AFB2: y= 1.7786 x, R2 = 0.9892; AFG: y= 1.7607 x, R2= 0.9969; and AFG2: y= 0 1.2411x, R2= 0.9986. All the curves were correct, with a regression coefficient of R2 > 0.9892.

Recovery percentage: The ranges of the recovery percentage were AFB1 (83%), AFB2, 75%, AFG1 (96%), and AFG2 (81%). These data were considered in the sample concentration calculations.

Limits of Detection (LOD) and Quantification (LOQ): The LOD were AFB1 (0.1 ng), AFB2 (0.01 ng), AFG1 (0.01 ng), and AFG2 (0.5 ng). The LOQ depends on the LOD and is multiplied by a factor of 5; therefore, the LOQ were AFB1 (0.5 ng), AFB2 (0.05 ng), AFG1 (0.05 ng), and AFG2 (2.5 ng).

Statistical Analysis

The results of the AF analysis of the 52 chili pepper sauces are shown in Table 1. The eight groups of chili sauce types were analyzed and compared using a Kruskal-Wallis test, and showed no significant differences among the samples with respect to the AFt amounts. The removal of all the AFG1 mutagenic levels (> 10 µg kg-1) [30] from the

analysis did not change the results. The summary statistics of all types of chilies are shown in Table 2.

No.	Sauce type and ingredients	Aflatoxin (µg kg-1)				Total AF (µg kg-1)
		AFB1	AFB2	AFG1	AFG2	
I Smoked "Chipotle", "Meco" and "Pasilla" chilli sauces						
1	'Chipotle' smoked and red Jalapeño peppers chilli sauce	0	0	0.35	2.76	3.1
2	Cheddar cheese, Chipotle peppers, corn starch, spices and condiments.	1.54	0	0	0.92	2.5
3	'Chipotle' smoked and jalapeño chilli peppers, vegetable oil, corn starch, spices	0.5	0.03	1	0.43	2.0
4	Ground smoked 'Chipotles' and jalapeño pepper, spices, starch, garlic	0.39	0	0.75	0	1.1
5	'Chipotle' smoked chilli peppers	0.85	0	1.29	0.63	2.8
6	'Chipotles' smoked and ancho chili peppers and spices	0.37		0	0	0.4
7	'Chipotle' smoked chili pulp.	0.26	0	3.96	0	4.2
8	'Chipotle' smoked sauce sunflower seed oil, paprika, spices	0.24	0	6.92	0	7.2
9	Hot 100% 'Chipotle' smoked sauce and spices	0.3	0	13.29	1.18	15.0*
10	'Chipotles' smoked and ancho chili peppers, garlic, spices, oil sauce	0.35	0	4	0	4.4
11	Autentical 'Mecos' chilies (smoked)	1	0	4.17	4.01	9.2
12	Black sauce. Pasilla chili pepper, spices.	2.39	0	0.96	0	3.4
13	'Pasilla' chili sauce soybean oil, spices,condiments	0.2	0	1.42	1.45	3.1
II Fresh "Jalapeño" peppers						
14	Green Mexican sauce. Jalapeño peppers.	0.28	0	5	0.86	6.1
15	Jalapeño chilli slices, spices and condiments	0.46	0	4.17	0	4.6
16	'Jalapeños' ground chilies peppers, vegetable oil, starch, powdered garlic	2.61	0	1.92	0	4.5
17	'Jalapeños' chili ground peppers, vegetable oil, starch, powdered garlic.	1.33	0	13.71	0	15.0*
18	Green 'Comaleña' Jalapeño pepper sauce, modified starch, powdered garlic.	0.59	0	0	1.25	1.8
19	'Jalapeños' ground, Jalapeño peppers.	0.33	0	0	0	0.3
20	Pungent sauce, Jalapeño peppers.	0.5	0	0	0	0.5
III Fresh "Serrano" pepper sauces						
21	Hot chili sauce, dry Serrano peppers, spices	0.41	0	2.63	0	3.0
22	Home made Mexican type. Serrano pepper.	0.63	0.02	0.79	0.25	1.7
23	Home made green sauce. Serrano pepper	0.22	0.01	0.5	0.61	1.3
24	Pungent sauce (Yellow label) dry puya and serrano peppers, spices.	0.96	0	4.58	0.79	6.3
25	Ground ("Molcajeteada") sauce. Serrano peppers	0.33	0.01	2.58	0.57	3.5
26	'Macha' sauce dry Serrano chili peppers.	0	0	5.83	0.99	6.8
IV Dry "Morita" chilli sauce						
27	Home made Northern sauce, Morita chili pepper, powdered garlic, spices.	0	0	0	0	0
28	Taco sauce morita chili pepper, powdered garlic, spices	0.28	0	0.67	0	1

29	Light sauce. Morita pepper, spices.	0.44	0	0.63	0	1.1
V Pungent “Habanero” chilli pepper sauce						
30	‘Habanero’: Extra pungent chili pepper, powdered garlic,	6.52	0	9.62	0	16.0*
31	‘Habanera’ Brava sauce. Red & yellow habanero peppers, spices.	0.18	0.04	0.18	0.51	0.9
32	‘Habanera’ red sauce Habanero chili pepper, olive oil, spices, garlic.	0.61	0	0	0	0.6
33	‘Habanera’ sauce. Habanero chili pepper, olive oil, spices, garlic.	0.67	0.07	0	0	0.7
34	‘Habanera’ Diabla sauce. Habanero chili pepper, olive oil, spices, garlic.	0.39	0	0	0	0.4
35	‘Habanero’ chili pepper sauce	0	0	20.83	0	21.0*
36	‘Habanero’ chili pepper sauce, condiments.	2.02	0.39	0	0	2.4
VI Aged pungent red peppers (“árbol”, “guajillo”, “piquín”)						
37	‘Piquín’ chilli pepper,condiments and spices,	0	0.01	0	0.31	0.3
38	Pungent , mix of chili peppers, spices, dyes	0.22	0.17	17.08	0	17.0*
39	Extra Hot. Aged red peppers.	1.07	0	42.2	0	43.0*
40	Hot sauce. Aged red peppers.	0.33	0	6.67	0	7.0
41	Very pungent (Black label), dry ‘árbol’ chilli peppers, condiments, spices.	0.46	0	0	1.58	2.0
42	Hot chili sauce red peppers (árbol & guajillo), spices.	3.04	0.03	30	2.96	36.0*
43	Hot chilli sauce red tree pepper, condiments, spices,	0.46	0.01	0.42	0.5	1.4
44	‘Tabasco’, vinegar, red chili pepper, salt	0	0.04	0	2.57	2.6
45	Classic sauce. Guajillo pepper, condiments.	0.42	0	0	1.18	1.6
46	Pungent sauce (very hot) dry árbol chili peppers, condiments, spices.	0.61	0	7.08	0	7.7
47	‘Árbol’ and ‘piquin’ chili peppers sauce, spices.	1.26	0	0	0	1.3
VII “Chamoy” chilli sauce						
48	‘Chamoy’ sauce, Guajillo pepper	0	0	0	0	0
49	‘Chamoy’, modified starch, chili pepper mix, flavoring.	1.2	0	114.4	0	116*
VIII Different not determined types of chilli sauces.						
50	Hot Sonora sauce dried peppers, spices	5	0.17	1.72	0	6.9
51	Clásica, ground chili peppers	0	0	6.25	0.79	7.0
52	Hot sauce for snacks. Dry chili peppers	0.35	0	7.08	1.71	9.1

Table 1: Types and concentration of aflatoxins in chili sauces in Mexico, *Mutagenic amount of aflatoxin (≥ 10 µg kg-1).

Aflatoxin	Number	Mean	Median	SD	Minimum	Maximum
AFB1	52	0.8186538	0.44	1.209672	0	6.52
AFB2	51	0.0196078	0	0.0634022	0	0.39
AFG1	52	6.627885	1.29	17.19165	0	114.4
AFG2	52	0.5540385	0	0.8937084	0	4.01
Descriptive statistics taking out the AFG1 values ≥ 10 µg kg-1						
AFG1	45	2.069778	0.75	2.638502	0	9.62

Table 2: Descriptive statistics considering the four Aflatoxins of all kinds of chili pepper.

A Kruskal-Wallis test, which uses ranges of sampling data from three or more independent groups, was applied to the data. This test is used to confirm the null hypothesis that the independent samples were derived from distributions with the same medians. The alternative hypothesis is that groups have different medians, and therefore different distributions. It is an alternative to analysis of variance

(ANOVA) when one or more of the assumptions are not met. In this particular case the assumption of Normal distribution is not realistic, in fact the data are not symmetrical and the use of non parametric test are recommended.

A Kruskal-Wallis chi-squared test was conducted to detect differences among the aflatoxins, and the value of the statistic was 55.889 with a significant p-value of less than 0.001. Given the mutagenic amount of AFG1, we excluded values of AFG1 > 10 μg kg-1 from our analysis, which yielded a Kruskal-Wallis statistic of 48.64 with a significant p-value less than 0.001 (Table 2).

Therefore, we performed the Wilcoxon rank sum test of pairs of aflatoxins, revealing no significant difference of AFB1 and AFG1 and significant differences for all other pairs. The Wilcoxon statistics for pairwise comparison were not significant (0.05).

Other common ingredients in chili pepper sauces were xanthan gum, spices, natural red coloring, 0.05% sodium benzoate, artificial colors FD&C red No. 40 and yellow No. 5, 0.05 to 0.1% sodium benzoate and 0.05% potassium or sodium sorbate as preservatives, potassium sugar, condiments and herbs, monosodium glutamate, and artificial EDTA with unknown effects for the AF analysis. Notably, the AFs in chili pepper sauces were not diminished in comparison to those observed in raw dry chili peppers in Mexico [5].

The AF-producing fungi Aspergillus have been isolated from samples of red peppers of the genus Capsicum [31,32] and in Mexican samples in the present study.

The pungent compounds of the Capsicum fruit are called capsaicinoids (capsaicin and its analogs), including capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. These capsaicinoids are the major components of most Capsicum species, constituting approximately 95% or more of the total capsaicinoid content [33]. In C. annum the proportions detected through mass spectrometry are capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) [34]. Thus, a discussion on capsaicin is useful to explain these results.

A study in rats found the feeding of chilies to be the determining tumorigenic factor [35]. However, there is no reference to the presence or amount of AFs in relation to this proposed carcinogenic effect of chilies, and so these rats could have been fed chili peppers with AF levels that are considered to be contaminated.

The capsaicin in red pepper increases cancer occurrence as a promoter [36]. However, no AF analysis has been conducted on the chilies used in these studies; thus, it remains unknown whether the capsaicin was pure or contaminated with AFs. Some reports [37] have suggested that red chili induces profound alterations in lipid metabolism as a prerequisite for colon carcinogenesis, showing that the incidences of intestinal tumors/polyps in rats were 0/30 (negative control), 27/30 (positive control), and 25/30 (red chili powder), although the effects on lipid metabolism were considerably overcome when coconut kernel was included in the diet. Indeed, AFs primarily affect lipids in the liver, causing fat liver, cirrhosis and hepatocarcinomas, but are also involved in colorectal cancer tumors [38]. The tumor incidence (intestine and colon) showed that chilis not only promote carcinogenesis but, in the presence of a carcinogen, also accelerate the process [39]. Carcinogenicity/tumorigenicity studies have reported that chilies in the feed are the determining tumorigenic factor for cystic cholangiomas, solid cholangiomas, adenocarcinomas, and hepatomas [35]. No tumors were observed in control mice [40]. However, these studies did not analyze the AFs in the chilies.

The carcinogenic, co-carcinogenic, anti-carcinogenic, anti-tumorigenic, tumor promotion, and anti-tumor promotion effects of capsaicin have been reported in animal studies [21]. Chili not only promotes carcinogenesis but also, in the presence of a chemical procarcinogen, e.g., 1, 2-dimethylhydrazine (DMH), accelerates the process [37,39]. The co-carcinogenicity of red chili has been demonstrated, and red pepper alone may not be carcinogenic to the stomach, but may enhance N-methyl-N′-nitro-N-nitrosoguanidine [MNNG]-induced carcinogenesis over a prolonged period of time. Red pepper promotes duodenal carcinogenesis, but has no cancer-promoting effect on the glandular stomach [41]. In acute oral toxicity studies, capsaicin induced gastric fundal hemorrhage in some of the animals that died [42]. AFs induce hemorrhages, and some studies have reported that pH changes in digestion might mitigate the effects of AFs on the induction of stomach cancer [43]. Surh and Lee (1996) [44] proposed a mechanism for the carcinogenic, co-carcinogenic, and anti-carcinogenic effects of capsaicin. These authors argued that although a minute amount of capsaicin displays few or no deleterious effects, excessive ingestion of this compound has been associated with necrosis, ulceration, and even carcinogenesis. They further noted that cytochrome P450-dependent mixed function oxidases metabolize capsaicin to reactive species [45-47], which may subsequently interact with target cell DNA in an irreversible manner, thereby triggering mutagenicity and malignant transformation. Capsaicin acts as a tumor promoter for the development of diethylnitrosamine-initiated enzyme-altered foci in the livers of male Sprague-Dawley rats [48]. These properties are also present in the AFs of chili peppers. The same phenomenon has been observed with capsaicin promoting the carcinogenic effect of AFs, although there was no analysis of the AFs in the Capsicum.

In contrast, a lack of tumor-promoting activity has been reported for capsaicin, a principal pungent ingredient of red pepper, in mouse skin carcinogenesis suggesting that that capsaicin lacks the ability to promote tumors in mouse skin [49].

Capsaicin was not mutagenic or had a weak mutagenic response in the Ames test [50-53]. Capsaicin increased bone marrow cells in rats [51, 54], but was not mutagenic [55]. In fact, AFs in natural foods are also not mutagenic, thus, these results were consistent. Many phytochemical compounds in vegetables and fruits block carcinogenic and mutagenic processes. In maize, linoleic acid could block the mutagenicity of AFB1 as an antioxidant, thereby protecting Capsaicin cream also showed good results for the treatment of patients with notalgia paresthetica, who presented with symptoms of intermittent intense itch or pain, burning, stinging, coughing, and erythema of the back, followed by burning and swelling of the face, resembling an urticarial reactionthis vegetable and indirectly protecting humans who are exposed to high quantities of AFs in the diet. In chili peppers, capsaicin could block the mutagenicity according to the Ames test [56, 57].

Topical capsaicin was applied in the treatment of postherpetic neuralgia [58], but the effect of this compound on the acute flare that was induced by the antigens was inconsistent and failed to reach statistical significance [59]. Topical capsaicin cream is safe and effective for the treatment of painful diabetic neuropathy [60].

Patients with psoriatic lesions are unresponsive to low capsaicin concentrations [61]. Capsaicin cream also showed good results for the

treatment of patients with notalgia paresthetica, who presented with symptoms of intermittent intense itch or pain, burning, stinging, coughing, and erythema of the back, followed by burning and swelling of the face, resembling an urticarial reaction [62].

Capsicum annuum and C. frutescens-derived ingredients, including capsaicin, have been used in 19 cosmetic products at concentrations as high as 5% in tonics, dressings, hair-grooming aids, tonics for neck, hands and face, bath and hair preparations, tinctures, shampoos, fragrances, external analgesics, skin conditioning agents, etc. [63]. Furthermore, paprika (the ground dried pod of mild capsicum [Capsicum annuum L.]) can be safely used for food coloring in amounts consistent with good manufacturing practices [21].

A survey of AFs in herbs and spices was carried out in UK and additional cooking experiments were conducted to assess the stability of AFs in spiced sauces. Among 157 retail samples, including curry powders, pepper, cayenne pepper, chili, paprika, ginger, cinnamon and coriander, nearly 95% of the samples contained less than 10 μg kg-1 total aflatoxins (AFt), and only nine samples had higher levels. The highest concentration in a retail sample was 48 μg kg-1 in a chili powder. Only two samples, both chili powders, showed levels above 10 μg kg-1, containing 35 and 51 μg kg-1 AFt. Cooking experiments showed that aflatoxin levels in spiced sauces were not reduced through domestic cooking with either microwave or conventional gas oven heating [64].

Fungal infection and aflatoxin (AF) contamination were evaluated in dried hot chilies obtained from the African markets of Benin, Togo and Mali in 2006. Hot chili showed a high incidence of fungal contamination compared with other dried vegetables. Species of Aspergillus were dominant on all marketed dried vegetables, regardless of country. Hot chilies containing AFs (3.2 μg kg-1) were naturally contaminated with AFB1 and AFB2 [65]. The AFs from 64 compound samples of 3 types of dried chilies obtained from 48 markets in Mexico City were analyzed for AFt in "Guajillo" (AFt 0.92 μg kg-1), "Ancho" (AFt 3.49 μg kg-1) and "Piquín" (AFt 3.14 μg kg-1) chili peppers [5].

As previously mentioned, AFs have been implicated as risk factors for hepatocellular carcinoma in areas of the world with a high incidence of this tumor. The use of aflatoxin-albumin adducts in peripheral blood was validated as a measure of individual exposure to this carcinogen in Gambia. All subjects were exposed to an average daily intake of 1.4 mg/day. A significant correlation ($r = 0.55$; $P = < 0.05$) was observed between the dietary intake and the level of albumin-bound AF. A comparison of the matched chronic hepatitis B surface antigen carriers with noncarriers did not reveal any difference in adduct formation for a given dietary intake of AF. These studies demonstrate the validity of aflatoxin-albumin adducts as markers of human exposure to this carcinogen [66].

Although the body is constantly removing mutations, aflatoxins primarily bond with guanine in DNA, leading to the accumulation of these compounds over time. As the accumulation increases, the risk for developing cancer increases. All of these data suggest that the amount of aflatoxins accumulated in the body can affect health.

However, the best way to eliminate aflatoxins is to ensure that chilies are stored in proper conditions. Chilies become contaminated with AFs when stored in conditions of humidity and at warm temperatures, which promote the growth of Aspergillus fungi. Distributors should assess the conditions in which the chilies are stored prior to using these vegetables in their products. Furthermore, the government has a responsibility to ensure the safety of individuals, and should therefore work on solving these issues.

In the present study eight chili pepper sauces (15%) from the different groups surpassed the 10 ppb AFt tolerance limit of the Codex Alimentarius and had 15 to 116 μg kg-1 AFt. The average AFs from the 52 samples were 0.89 μg kg-1 for AFB1, 0.02 μg kg-1 for AFB2, 6.63 μg kg-1 for AFG1, 0.55 μg kg-1 AFG2 and 3.69 μg kg-1 for AFt, which were all within the globally accepted limits.

Because the chilies for one batch of salsa might be stored differently than those from another, one batch of salsa might be acceptable, while another batch might contain AFs. Nevertheless, chili sauces can be contaminated with AFs, contribute to the mutagens that are ingested in food accompaniments and should therefore be consumed with caution. Thus, these results suggest that there is no consistent basis for warning the public about AFs in the most commonly used salsas, but their use should be moderated because many are not safe to eat.

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