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Identification and Quantification of Aflatoxins, Carcinogenic Toxins from *Aspergillus* Spp. Fungi in Almonds (*Prunus Dulcis* (Miller) D.A. Webb)

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ABSTRACT

The almond (*Prunus dulcis*) is an oilseed that is consumed fresh or processed. Aflatoxins (AFs) are fungal secondary metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus* that have adverse effects on human and animal health. The International Agency for the Research on Cancer classifies aflatoxins in Group 1 as proven human carcinogens.

The purpose of this research was to identify and quantify aflatoxins in 35 almond samples from three markets in Mexico City. The analytical method was validated, and the quantification was performed via liquid chromatography.

Eight aflatoxins were identified: four basic aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) and four hydroxylates, aflatoxins M_1 (AFM_1), M_2 (AFM_2), and P_1 (AFP_1) and aflatoxicol (AFL).

The 35 samples were contaminated with AFs, with an average total aflatoxin concentration of 273.22 ng g^{-1} and with at least one of the eight AFs present: AFB_1 in 80% of the samples (28/35); AFB_2, 89% (31/35); AFG_1, 74% (26/35); AFG_2, 83% (29/35); AFM_1, 40% (14/35); AFM_2, 91% (32/35); AFP_1, 83% (29/35); and AFL, 86% (30/35). There were significant differences only among the hydroxylates, with AFM_2 significantly differing from all the others.

The amount of total aflatoxin in the almond samples, including the hydroxylated metabolites, represents the real ratio during ingestion and is a risk to the consumer and an important contributor of food carcinogens in the diet.

Keywords: Oilseeds, Aflatoxins, Contaminants, Carcinogens, Food toxins

INTRODUCTION

Almond (*Prunus dulcis* (Miller) D.A. Webb. (syn. *Prunus amygdalus*, L., Batsch) is a dicotyledonous drupe of the Rosaceae family, a tree adapted to harsh climates and poor soil quality [1], but it requires large amounts of nutrients and has an extensive root system that allows it to be cultivated in a wide variety of ecological niches. Almond trees require cross-pollination to promote genetic variability and adaptability to new environments [2].

There are two types of almonds, sweet and bitter; almonds have low water contents (4 to 6%) and high levels of protein (18%), fat (54%), and carbohydrates (20%) and are rich sources of oil [3,4]. Almonds are rich in calcium, phosphorus and chemical components that strengthen bones and prevent osteoporosis, and can be used as milk substitutes for vegans and lactose-intolerant people, and they have applications in cosmetics [5,6].

In 2024, the size of the almond market was estimated to be 10.09 billion USD, and in 2025, the estimation of global almond production will reach 1.27 million metric tons [7]. California, in the USA, is the world's largest almond producer, producing 77% of the world's almonds, followed by Spain, Australia, Iran and Morocco. Mexico is an importer of almonds, not a producer country.

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Aflatoxins

Aflatoxins (AFs) are bis-dihydrofuran-coumarins, a group of toxic and carcinogenic secondary metabolites produced mainly by the molds *Aspergillus flavus, A. parasiticus, A. nomius, A. pseudotamarii* and *A. bombycis* [8] via a polyketide pathway [9].

In this study, four basic AFs, B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), and four hydroxylated metabolites, M1 (AFM1), M2 (AFM2), P1 (AFP1) and aflatoxicol (AFL), were analyzed (**Figure 1**). AFM1 and AFM2 are oxidative metabolic products of AFB1 and AFB2, and AFL is a reducing metabolite of AFB1.

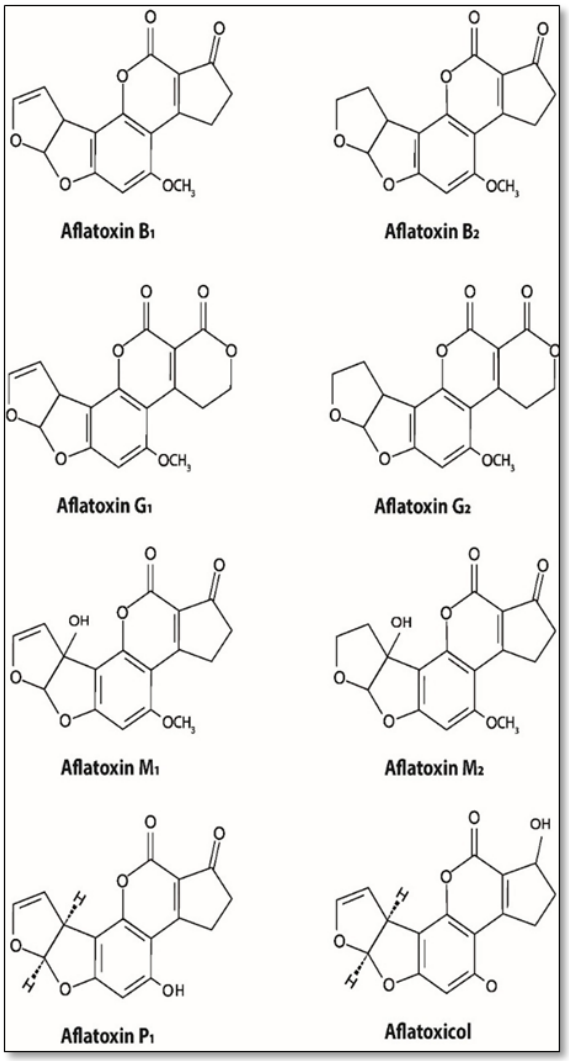

Figure 1. Aflotoxics.

AFs are potent mutagens, carcinogens, cancer biomarkers and teratogens that cause aflatoxicosis with acute effects when consumed in nanogram quantities, with symptoms including hemorrhage, immunosuppression, diarrhea, liver damage, edema, alteration in digestion and food metabolism, and death. The International Agency for Research on Cancer (IARC) classified AFB1 as Group 1, a tested human carcinogen [10], whereas AFM1 is classified as Group 2B, a possible human carcinogen [11].

Almonds are susceptible to AF contamination, and tolerance limits for AFs have been established to protect human and animal health; these limits vary across countries and institutions [12].

The structure and physicochemical properties of AFs have been reported [13], have been shown to resist cooking, fermentation, pasteurization, ultra-pasteurization, and nixtamalization. However, they can be destroyed via drastic treatment, such as autoclaving in the presence of ammonia or hypochlorite [14].

Chronic poisoning due to the consumption of traces of AF - contaminated food for weeks, months or years is a frequent situation. A synergistic effect between exposure to AFs and

malaria, kwashiorkor or protein malnutrition has been observed [15].

AFB1 is the most toxic; it is transported by red blood cells and plasma proteins to the liver, enters in the cells and is metabolized to the hydroxylates AFM1, AFP1, AFQ1 and AFL [16], which are considered AF-decomposed excretion products. AFB1 can also form an unstable oxidized, electrophilic product called AFB1-8,9-epoxide that binds to nitrogen 7 (N^7) in the guanine residues of DNA and proteins to form the adduct 8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B1 (AFB1-N^7-Gua), which is responsible for the mutagenic and carcinogenic activity of AFB1 because it interrupts transcription and decreases ribonucleic acid synthesis [17].

AFs are regulated in more than 75 countries (with limits expressed in µg kg^{-1} or ng g^{-1}). Since 1998, European Commission (EC) regulations have established AFB1 and AFt limits of 2 and 4 µg $kg^{-1,}$ respectively, in almond [18].

The purpose of this study was to identify and quantify AFs (AFB1, AFB2, AFG1, and AFG2) and them hydroxylates (AFM1, AFM2, AFP1 and AFL) from fresh almonds purchased from the three most representative and important markets in Mexico City.

MATERIALS AND METHODS

The validation of the extraction and quantification method by high-performance liquid chromatography (HPLC) was the first step.

The analytical method was validated with the parameters of linearity, detection and quantification limits, recovery percentage and selectivity [19]. Analytical methods based on cleaning with immunoaffinity columns and HPLC with derivatization and fluorescence detection are used in the quantification of AFs in food.

Sampling

Thirty-five representative samples of 100 g of fresh almonds were purchased from three of the most representative markets in Mexico City (**Figure 2**)

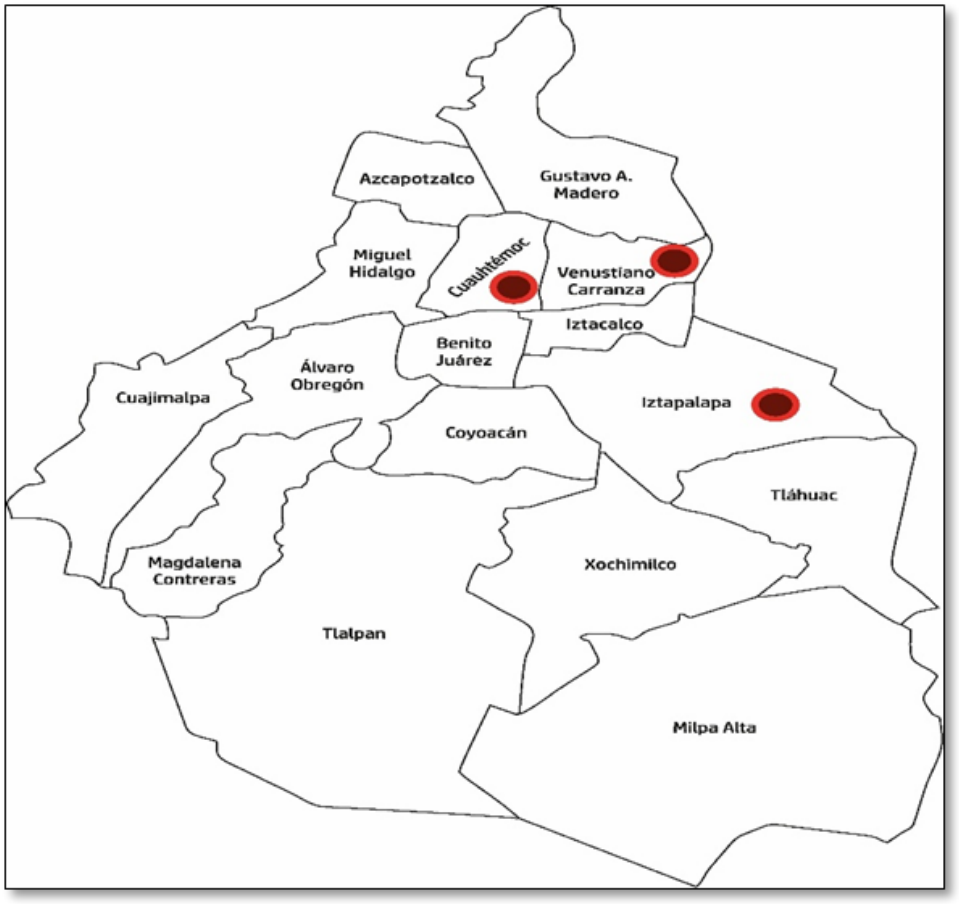

Figure 2. Sampling points for almonds in Mexican markets.

Central de Abastos. Eje 6 Sur, Col. Central de Abasto, Alcaldía (Town Hall) Iztapalapa, 09040 CdMx.

San Juan. Calle Ernesto Pugibet 71 Col. Centro. Alcaldía (Town Hall) Cuauhtémoc, 06010 CdMx).

Jamaica. Avenida H. Congreso de la Unión, Col. Jamaica. Alcaldía (Town Hall) Venustiano Carranza, 15800 CdMx).

These markets import almonds from different origins, with great diversity. There have been no official data since 1991 regarding almond production in Mexico however, according to USDA, Mexico imported 10,000 tons of almonds with shells from the United States, which ranks first in exports of this nut [20]. Mexico City receives all of its almonds from abroad.

Chemical extraction of AFs

Fifty grams of sample and 5 g of sodium chloride (J.T. Baker NaCl) were blended (Waring Commercial Blender, Mod. 7010S) with 100 mL of distilled water and 150 mL of HPLC-grade methanol (J.T. Baker MeOH), and the mixture was ground at high speed for 2.5 minutes to obtain a homogenate, which was then centrifuged (ALC 4235 Cool Working System, Milan, Italy) at 4000 rpm for 10 min, after which the supernatants were recovered.

For extraction, total aflatoxin (R-Biopharm Rhône Ltd., UK) immunoaffinity columns were used to purify one gram of sample [21]. The immunoaffinity columns contained total anti-aflatoxin antibodies, with a recovery percentage of 97 to 100%.

To increase and homogenize the AF fluorescence, the sample eluates were derivatized via the methods of Akiyama [22] and Kok [23]. Finally, the proteins were quantified in triplicate via liquid chromatography.

The quantification of the samples was carried out on an HPLC instrument (Series 1200) with an isocratic pump (G1310A), fluorescence detector (G1321A) and autosampler (G1329A), all from Agilent Technologies, with an Agilent Eclipse XDS - C18, 4.6 × 250 mm chromatographic column of 5µm particle thickness. The software used for HPLC was Chem Station 32.

The conditions of analysis were as follows: H_2O/ACN/MeOH mobile phase (65:15:20 v/v/v); 60 µL injection; flow, 1 mL min^{-1}; analysis time, 30 min; excitation wavelength, 360-362 nm; and emission wavelengths, 425 nm for AFB1, AFB2, AFM1 and AFL and 450 nm for AFG1 and AFG2 [14].

Validation of the method

The validation was carried out in accordance with the parameters of linearity (calibration curves), limits of detection (LODs) and quantification (LOQs), percentage of recovery and selectivity. The AF standards in their original vials were dissolved in 1 mL of benzene solution: acetonitrile (98:2 v/v).

a) **Linearity**: The stock concentration of 1 µg mL^{-1} of each AF standard was calculated on the basis of its absorbance, molecular weights and extinction coefficients, and twelve concentrations were obtained from each AF standard separately. A minimum of 4 points were chosen to construct the trend line, and the correlation coefficients were obtained using Excel [14,24].

b) **Limits of detection (LODs) and quantification (LOQs)**: The LOD is the minimum amount of analyte that can be detected, although it is not necessarily quantified with precision and accuracy. The LOQ is the concentration of analyte (AF)that provides a result 10 times greater than the noise signal [25], the minimum amount of analyte that can be quantitatively determined with adequate accuracy and precision.

c) **Recovery Percentage:** The recovery percentage is the amount of AF recovered from a sample added and subjected to **the** complete analytical method; it allows us to evaluate the efficiency of the entire method and is expressed as a percentage. One gram of almond was individually fortified with 50, 100 or 200 ng of AFB1, AFB2, AFG1, AFG2, AFM1, AFM2, AFP1 or AFL, and the complete method for mycotoxins was applied [24].

Selectivity

Selectivity represents the ability of the mobile phase with the stationary phase to separate a pair of peaks that elute very closely, which directly influences the resolution (*Rs*).

Statistical Analysis

The nonparametric Kruskal–Wallis test was performed to determine whether there could be potential differences in the content of AFs in the almond samples. The Wilcoxon range test was also carried out to determine which groups were significantly different, and a paired t test was performed to determine the relationship between AFB1 and AFL in the 35 samples.

RESULTS AND DISCUSSION

Validation of the method

The experimental data analysis revealed that the detection and HPLC quantitation methods for the eight AFs in the almond samples met the requirements for sample analysis. The results of the linearity, LOD and LOQ parameters are shown in **Table 1**.

To determine the linearity, the linear regression parameter and the correlation coefficient (R2) were used. **Figure 3** presents the calibration curves for each AF.

Recovery Percentage

The extraction method resulted in recovery percentages ranging from 85% to 100% for the eight AFs in the almond samples, so it was therefore considered efficient (**Table 2**).

Table 1. Detection limits for each aflatoxin in the linearity validation test.

Aflatoxin	LOD (ng g^{-1})	LOQ (ng g^{-1})	Recovery %	Retention time range (min)	R^2
AFB_1	0.5	5.0	100	7.085-8.849	0.9986
AFB_2	0.05	0.5	98	17.452-20.228	0.9817
AFG_1	0.5	5.0	85	7.681-9.541	0.9898
AFG_2	0.5	5.0	98	11.215-14.513	0.9946
AFM_1	0.05	0.5	100	2.920-3.024	0.9917
AFM_2	0.05	0.5	98	20.208-22.447	0.9946
AFP_1	0.05	0.5	98	15.563-19.318	0.9960
AFL	0.01	0.1	96	3.032-5.569	0.9978

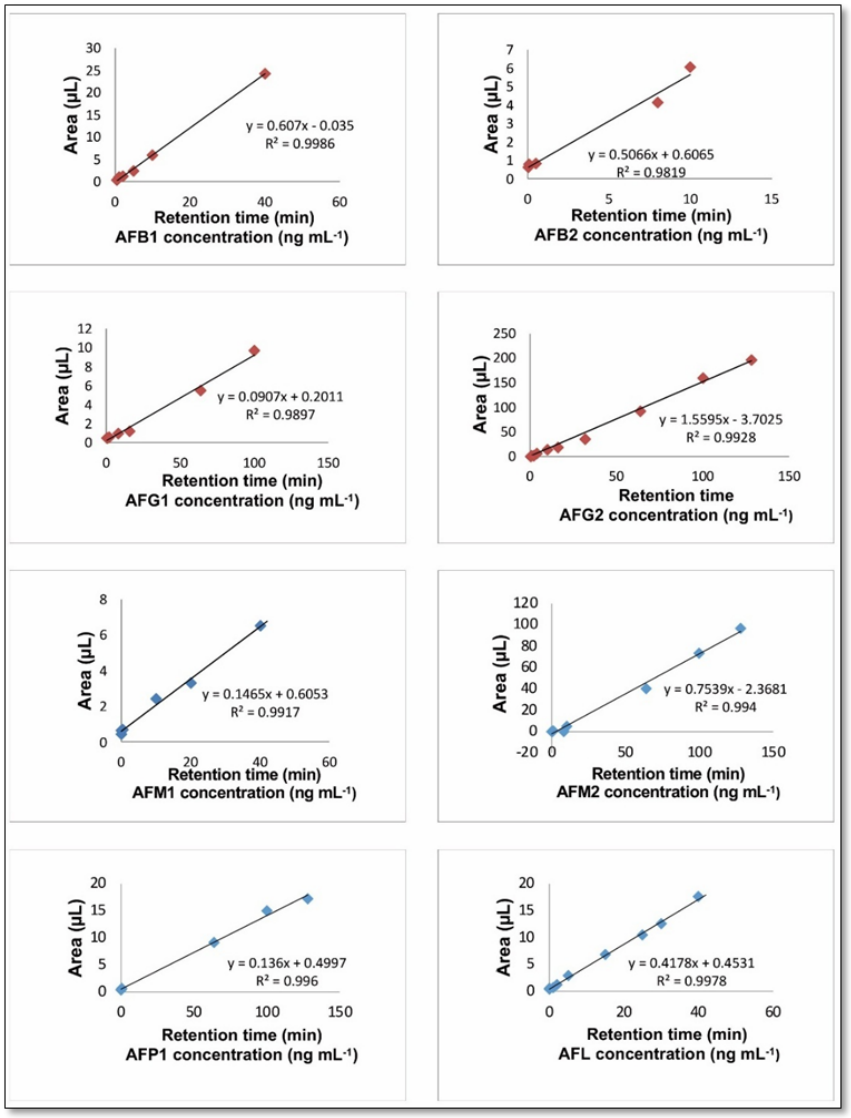

Figure 3. Calibration curves for each AF.

Table 2. Results of the recovery percentage experiment.

AF	Basal (ng mL^{-1})	AF (ng mL^{-1})	Added AF Area (µL)	Area (µL) of AF recovered	Recovered AF (ng mL^{-1})	Recovery %
AFB_1	0	100	60.79	60.66	99.78	100
AFB_2	0	100	60.8	59.51	97.87	98
AFG_1	0	100	9.68	8.27	85.48	85
AFG_2	0	50	63.03	61.76	48.99	98
AFM_1	0	50	7.5	7.49	49.93	100
AFM_2	0	200	116.48	114.39	196.4	98
AFP_1	0	50	7.11	6.95	48.87	98
AFL	0	50	22	21.14	48.05	96

Selectivity

The recovery percentage results agreed with the international acceptance criteria (EC, 2010) [18]. In almonds, the AFM1 and AFL peaks have the lowest pairwise chromatographic resolution (AOAC, 2005) [24]. The selectivity (α) of 1.338 indicates that the analytical method was efficient. Acceptable separation (Rs = 1.036) of the two components with minimal overlapping of the peak areas was obtained (**Figure 4**).

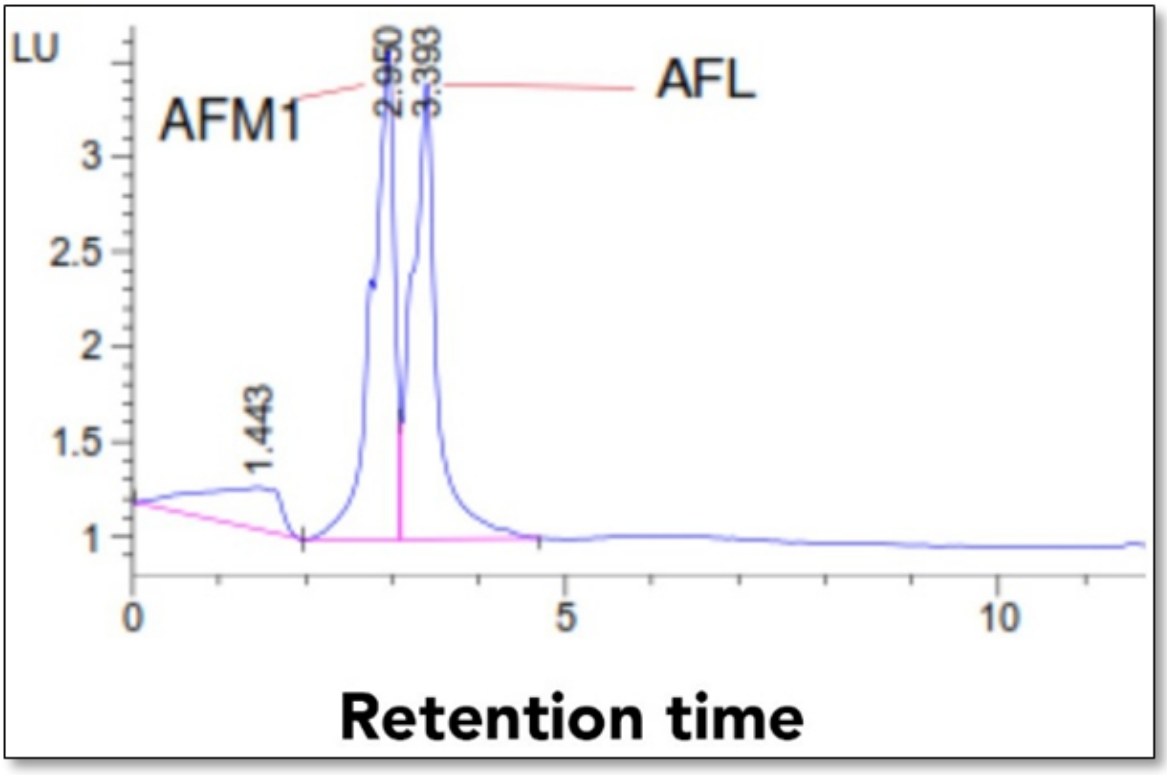

Figure 4. Chromatogram of sample 29 with AFM_1 and AFL with good selectivity and efficiency.

Aflatoxins in almond samples

The AFt concentrations in one gram of almond, as shown in **Table 3**, are high because they represent the sum of the basic AFs and the hydroxylated AF metabolites, representing the real ratios of AF ingested. The lack of reports of hydroxylated AF metabolites decreases the actual consumption ratios.

Table 3. Total AF concentrations (ng g^{-1}) in 1 g of almond.

Sample	Basic AF (ng g^{-1}) (%)				Hydroxylated metabolites (ng g^{-1}) (%)				AFt
	AFB_1 (80%)	AFB_2 (89%)	AFG_1 (74%)	AFG_2 (83%)	AFM_1 (40%)	AFM_2 (91%)	AFP_1 (83%)	AFL (86%)	
1	26.8	0	261.5	23.4	0	0	41.8	30.1	383.54
2	8.6	0	41.2	17.2	0	10.5	216.9	2.6	297.06
3	73.0	3.4	38.1	24.0	0	4.6	1.4	0	144.51
4	11.4	20.2	115.0	24.5	0.1	13.7	329.4	3.8	518.22
5	30.9	7.3	65.9	41.0	0.1	12.1	232.0	6.2	395.51
6	5.4	49.2	47.4	8.6	3.8	32.3	468.2	2.5	617.44
7	4.2	2.2	19.1	2.8	0	22.8	54.6	1.4	107.18
8	2.9	4.0	0.3	2.2	0	14.2	0.9	3.7	28.10
9	20.0	109.9	70.8	21.2	0	4.0	170.0	8.2	404.14
10	9.3	16.2	185.1	5.0	16.9	17.3	50.2	84.2	384.16
11	85.5	8.5	111.9	6.2	0	18.4	77.8	97.9	406.39
12	5.5	8.5	6.4	42.4	0	15.5	59.2	1.4	138.68
13	31.5	8.6	87.5	4.5	13.6	7.5	20.5	2.4	176.05
14	17.4	1.3	137.8	3.0	14.9	1.1	0.4	0.9	176.74
15	31.3	2.0	138.7	3.3	0	4.7	1.6	0	181.57
16	46.9	2.0	32.2	3.5	15.6	1.9	1.0	0	103.12
17	32.8	11.6	604.9	20.5	0	6.3	1.9	0	677.98
18	3.9	7.0	380.8	29.4	0	14.5	26.3	3.6	465.50
19	53.5	12.7	144.3	58.0	0.4	15.4	34.8	2.0	321.19
20	92.9	4.1	368.5	52.8	0	6.8	28.4	0	553.53
21	46.8	19.7	19.4	54.3	0.2	19.6	195.7	13.1	368.67
22	3.7	96.7	24.5	8.2	0	12.6	551.6	4.8	702.26
23	20.6	59.0	21.3	47.3	2.4	17.7	130.8	5.9	304.93
24	7.3	75.9	48.6	42.6	0.5	16.7	64.5	5.6	261.60
25	5.3	87.7	24.1	7.0	2.0	50.5	114.5	3.2	294.29
26	0	0.5	0	0	0	2.7	0.2	72.8	76.17
27	0.3	0.4	3.1	0.8	0	2.3	0.1	114.1	121.26
28	0	0.0	0	0	0	1.9	0	98.7	100.62
29	0	0.6	0	0.5	129.6	6.8	0	98.4	235.84
30	0	0.4	0	1.0	0	3.5	0	103.7	108.58
31	0.3	0.3	0	1.1	61.5	3.4	0	104.8	171.46
32	0	0.2	0	0	0	2.0	0	63.6	65.76
33	0.5	0	0	0	0	0	0	113.4	113.93
34	0	0.2	0	0	0	2.5	0.3	57.7	60.66
35	0	0	0	0	0	0	0.7	95.4	96.14
Average	19.38	17.70	85.67	15.89	7.47	10.40	82.16	34.46	273.22
Range	0.3- 92.9	0.02- 109.9	0.3- 604.9	0.5-58.0	0.1-129.6	0.1-50.5	0.1-551.6	0.9- 114.1	0.02-605

Note: 1 μg kg^{-1} = 1 ng g^{-1}

There are reports of hydroxylated metabolites synthesized by microorganisms and animal livers but not from the metabolism of almond seeds.

STATISTICAL ANALYSIS

The results of the Kruskal-Wallis tests are shown in **Table 4**.

Table 4. Kruskal-Wallis tests for Aflatoxins in almonds.

Aflatoxins	Degrees of freedom	Kruskal-Wallis Value	P value (Significance)	Significant difference
AFB1, AFB2, AFG1, AFG2	3	5.940	0.1146	No
AFM1, AFM2, AFP1, AFL	3	27.083	<0.0001	Yes
All	7	34.413	<0.0001	Yes

With Wilcoxon's rank sum statistical test corrected for multiple tests:

For the hydroxylates, AFM2 was significantly different from all others, with a value of $p < 0.0001$ in all cases. The rest were not significantly different from each other.

When all the different pairs of AFs were tested, only the levels of AFM2 were significantly different from those of all the other AFs (**Table 5**). The p values were as follows:

Table 5. Wilcoxon's Rank Sum statistical test and comparison of AFM2 among other AFs.

Against	AFB1	AFB2	AFG1	AFG2	AFM1	AFP1	AFL
AFM2 10.45	0.003	0.004	0.001	0.002	0.0006	0.0003	0.0007

Finally, a paired t test was performed to elucidate the relationship between AFB1 and AFL in the 35 samples, and no significant difference was found, perhaps because they are interconvertible AFs. When analyzing by source location, we found a significant difference in the San Juan Market, with a value of $p = 0.0014$. This is possible because the type of storage in the San Juan Market is smaller-volume boxes at retail. In Central de Abasto and Jamaica, wholesale volumes are sold with less care in their storage.

All 35 samples analyzed were contaminated with at least one of the eight AFs. U.S. export companies and Mexican import companies do not test AFs in almonds.

The San Juan market had a significant difference with respect to the other markets; the almond samples from this market had high concentrations of hydroxylated AFs with respect to the basic AFs, which did not exceed 1.72 ng g^{-1}.

Samples from the Jamaican market were the most contaminated by basic AFs (sample 17: 677.98 ng g^{-1}) and the most contaminated by hydroxylated AFs (sample 22: 702.3 ng g^{-1}). Among the 35 samples analyzed, sample 22 was the most contaminated, with 7 of the 8 aflatoxins present (AFM1 was absent), with an AFt concentration of 702.3 ng g^{-1} (**Figure 5**).

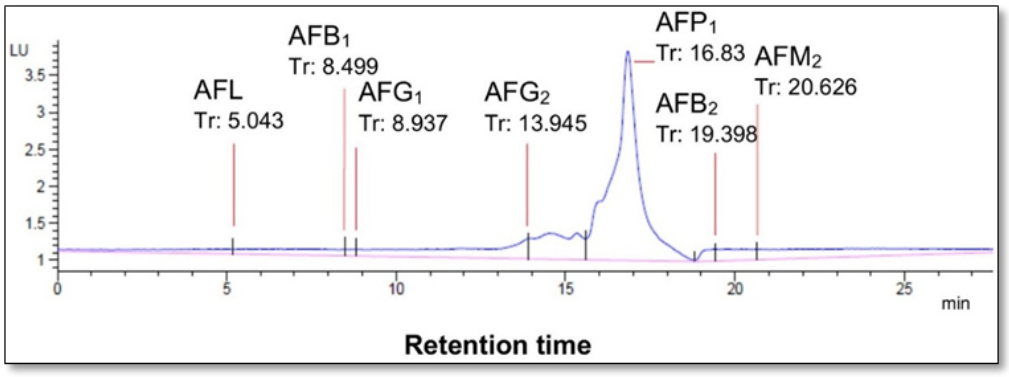

Figure 5. Chromatogram of almond sample 22, with an AFt concentration of 702.26 ng g^{-1} and identification of 7 of the 8 reported AFs.

Eight AFs, 4 basic and 4 hydroxylated metabolites, were found in the analyzed almond samples.

Statistical analyses indicated no significant differences between the basic AFs but significant differences among the hydroxylates, with AFM2 being significantly different from all the others.

The almond samples presented high AFt concentrations due to the inclusion of the eight aflatoxins, which are associated with foods and fats. Some species of *Aspergillus* obtain raw material for growth in a saprophytic form, and the secretion of hydrolases helps them utilize nutrients. The most important hydrolytic proteins associated with *Aspergillus* are proteases, amylases, lipases, and pectinases [26].

Lipases play a hydrolytic role in metabolism when triglycerides are used as a carbon source. The lipA gene, which encodes lipase, has been suggested to promote the synthesis of AFs in lipid-rich environments because of its apparent role in the capture of carbon nutrients from such sources [3,4].

The patterns of AF and lipid formation are similar. Under certain physical, chemical and biological conditions, the reduction of ketone groups is interrupted, condensation reactions are favored in the metabolic pathway for the production of fatty acids, and the synthesis of polyketone compounds (aflatoxins) occurs [27]. The biochemical pathway for AF biosynthesis involves approximately 23 enzymatic reactions, and most of the genes involved have been characterized [28]. Recent studies suggest that this process involves a complex pattern of positive or negative action affected by physiological responses due to internal and external stimuli. Transcriptional regulation of biosynthesis by *Aspergillus* sp. has been reported [29]. Norsolorinic acid is the first stable precursor of the biosynthetic pathway.

The formation of AFB1 and AFG1 from versicolorin A is derived from dimethyl-sterigmatocystin and versicolorin B, leading to the production of AFB2 and AFG2 [30]. *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *A. nidulans* produce sterigmatocystin, a penultimate intermediate in the AF synthesis pathway [31].

Aflatoxicol (AFL) is an AFB1 metabolite and is 18 times less toxic than AFB1. Aflatoxin P1 (AFP1) is a phenolic derivative resulting from the O-demethylation of AFB1, and its toxicity is considerably lower than that of AFB1. The formation of AFP1 represents a detoxification step. Aflatoxins M1 (AFM1) and M2 (AFM2) are oxidative metabolic products of AFB1 and AFB2 produced in the body after ingestion, with AFM1 being 2 to 10% less carcinogenic than AFB1. Although these hydroxylated derivatives have been studied and reported in animal models, their presence here has been verified in almond.

AF contamination in almond [3,4] is due mainly to *Aspergillus flavus* and, to a lesser extent, to *A. parasiticus,* regardless of the field of origin or state of storage during production [2,32]. In our case, the almond samples were probably infected with *A. parasiticus*, as it produces high levels of aflatoxins AFB1, AFB2, AFG1, and AFG2, which were found in all our samples, with AFG1 presenting the highest concentration of 604.90 ng g^{-1}.

Contamination of almonds with AFs can occur during fruit and seed development, during drying or storage, and after harvest; the risk of *Aspergillus spp*. infection and AF contamination can be controlled if adequate moisture conditions are maintained during storage [33]. Insect damage to fruits or seeds facilitates infection of the wounds and allows spores and propagules from the fungi to enter the seed. Insect damage is a key risk factor for AF contamination. The almonds analyzed did not present any external damage, so the high concentration of AF may be due to contamination of the fruit or to poor storage.

AFB1 and AFB2 have been detected in trace amounts in almonds from California, USA [34], and AFG1 and AFG2 have been detected at lower concentrations [35]. In California, postharvest almonds have a water content of less than 6% aw < 0.7 and withstand longer storage periods [3,4]. It is likely that a long storage period increases the AF concentrations once in Mexico.

Chemical compounds such as phenols, naphthoquinones, tannins and plumbagins, which have powerful effects on the biosynthesis of AFs, have only been found in trace quantities in the almond cuticle. The compounds with biological activity and that are produced in high concentrations in almond are triterpenoids, phenolics and sterols, but they have not been shown to have significant anti aflatoxicogenic activity. This may explain the high concentration of AFs in the samples analyzed. In almond, AFs tend to accumulate in the cuticle or integument [3,4].

Almonds are the food with the highest AF contamination among oilseeds, and the levels of basic AFs (139 ng g^{-1}) exceed the AF levels in peanuts (8 ng g^{-1}).

This study describes almond contamination with 8 AFs. The lack of AF control increases the AF concentration in almond.

CONCLUSION

Almonds exported from the USA to Mexico are among the foods most contaminated with AFs (139 ng g^{-1}), representing a risk to the consumer and a significant contribution of food carcinogens in the diet.

Almonds form the same hydroxylated metabolites following the same AF biosynthetic and decontamination paths as the animal liver does.

These new results show that almonds can be contaminated with eight aflatoxins, 4 basic aflatoxins (AFB1, AFB2, AFG1, and AFG2) and four hydroxylates (AFM1, AFM2, AFP1, and AFL).

There were good recoveries of > 85% for the eight AFs as well as good selectivity and chromatographic resolution.

All the samples were contaminated with at least one of the eight AFs.

None of the samples analyzed comply with the limits established by NOM-188-SSA1-2008 or by the *Codex Alimentarius* (2024) [36], and the highest concentration of AFt was 702.26 ng g^{-1}.

There were no significant differences between the basic AFs, but there were significant differences between the hydroxylates, with AFM2 being significantly different from all the others.

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