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# Carcinogenic Aflatoxins and Aflatoxicol in Cheeses Sampled in Mexico

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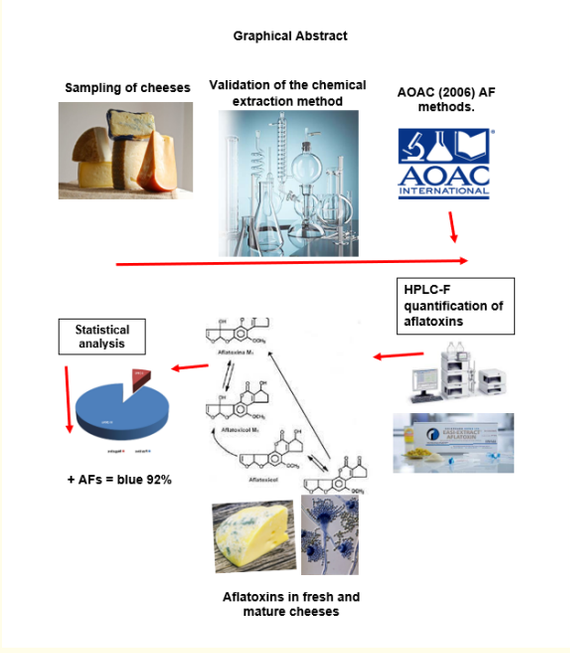
## Abstract

Fresh and mature cheese samples were collected in 2018 in Mexico City to identify aflatoxins ($AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$) and their hydroxylated metabolites ($AFM_1$, $AFM_2$, $AFP_1$, and aflatoxicol). Ninety two percent of the samples had aflatoxins, and aflatoxicol was the prevalent mycotoxin in 72% samples. and represent a health risk. Most of the $AFB_1$ was metabolized to aflatoxicol, which interconverts with $AFM_1$ to become a dangerous carcinogen. Aflatoxicol is a potent carcinogen that retains 70% of the mutagenicity of $AFB_1$. Aflatoxicol is a 'reservoir' for $AFB_1$ *in vivo*, prolonging its effective lifetime in the body, and is more potent toxin than $AFM_1$. There was no difference in AFs between fresh and mature cheeses and they are a health risk for humans due to the frequency of consumption. It is recommended to check aflatoxins in the ruminant feed.

***Keywords:*** *Aflatoxins; Carcinogens; Cheese; Hydroxylated Metabolites; Mycotoxins*

## Graphical Abstract



## Abbreviations

UNAM: Universidad Nacional Autónoma de México; AFs: Aflatoxins; $AFB_1$: Aflatoxin $B_1$; $AFB_2$: Aflatoxin $B_2$; $AFG_1$: Aflatoxin $G_1$; $AFG_2$: Aflatoxin $G_2$; $AFM_1$: Aflatoxin $M_1$; $AFM_2$: Aflatoxin $M_2$; $AFP_1$: Aflatoxin $P_2$; AFL: Aflatoxicol; DNA: Deoxyribonucleic; >: More Than; <: Less Than; LOD: Limit of Detection; LOQ: Limit of Quantification; AOAC: Association of Official Analytical Chemists; $R^2$: Coefficient of Determination; HPLC: High Performance Liquid Chromatography; nm: Nanometers; ng: Nanograms; ng $mL^{-1}$: Nanograms Per Milliliter; ng $g^{-1}$: Nanograms Per Gram; µm: Micrometers; mm: Millimeters; °C: Degrees Centigrades; ACN: Acetonitrile; mL/min: Millimeters/Minutes; P: Probability; AFL/DNA Adduct: Aflatoxicol/Deoxyribonucleic Adduct; pH: Hydrogen Potential, Measure of Acidity or Alkaline Solution; JECFA: Mix Committee FAO/OMS of Experts in Food Additives; Oltipraz: Agent that Modifies Carcinogen Metabolism by Inducing Phase I or II Enzymes. Synthetic Dithiolethione with Potential Chemopreventive and Anti-Angiogenic Properties

## Introduction

Worldwide cheese production and consumption, is expected to grow to $211.02 billion USD in 2022, at a compound annual growth rate of 9.4% [1]. The European Union, mainly Germany, France, United Kingdom, Holland and Poland, produced 37.1% of cheese worldwide (>10 million tons). USA was the second largest cheese producer (6 million tons). The cheese industry uses 67.5% of milk produced [2].

Cheese consumption per capita in Mexico is 4.3 kg [3], and in the European Union, it is more than 19 kg [4]. The cheese industry in Mexico, in 2018, produced 65,942 tons. The cheese types were double cream (15.7%), panela (12.0%), yellow (11.7%), Chihuahua (10.1%), Manchego (9%), string cheese (6.1%), and others (7.7%) [5].

Aflatoxins (AFs) are toxic secondary metabolites mainly from the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius* that contaminate several foods such as cereals, oilseeds, spices and dry fruits in the field, during storage and in derived products [6].

The ingestion of AFs by animals and humans causes different negative effects, such as immunodepression, hemorrhage [7], vomiting, hepatitis, cirrhosis [8], Reye syndrome, different cancers of the digestive system, cervical cancer [9], lung cancer [10], renal damage [11], dwarfism [12] and death.

AFs are recognized as potent mutagens and are classified as grade I carcinogens in humans [13]. Pregnancy can modulate both phase I and II metabolism and alter the biological potency of $AFB_1$ and increases DNA damage in mouse liver.

Pregnancy may constitute a critical susceptibility period for maternal health, and provide insight into the biochemical factors that could explain the underlying risks [14]. The metabolism of Aflatoxin $B_1$ ($AFB_1$), including epoxidation, inflammation, oxidative stress and DNA adduction, concerns to the initiation of cancer as well as $AFB_1$ exposures in occupational settings [15].

The toxic effects and population risk, that the exposure to $AFB_1$ represents, are demostrated in Mexican [16] and Brasilian reports [17], as well as the current need to create a governmental program that directly influences the control of the sources of exposure in order to reduce them.

$AFB_1$ and aflatoxin $B_2$ ($AFB_2$) are transformed in the livers of mammals to hydroxylated AF metabolites, aflatoxin $M_1$ ($AFM_1$), aflatoxin $M_2$ ($AFM_2$), Aflatoxin $P_1$ ($AFP_1$) and aflatoxicol (AFL). These AFs are present in 1-3% of milk samples, and can also be present in cheese [18] (Supplementary figure 1).

## Purpose of the Study

The purpose of this study was to identify and quantify AFs and their hydroxylated metabolites in fresh and mature, imported and national cheeses consumed in Mexico.

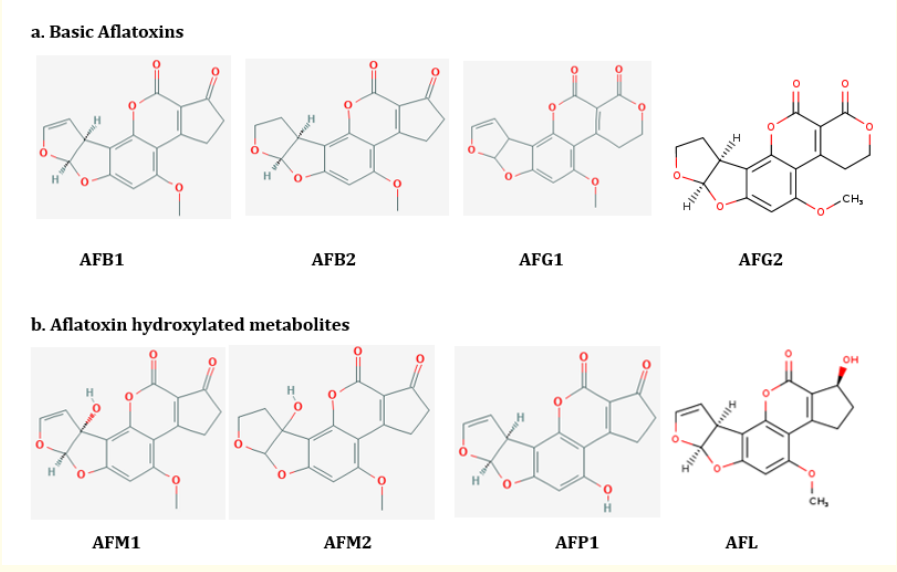



***Figure S1:*** *Chemical structure of aflatoxins.*

*a. Chemical structure of basic aflatoxins. Aflatoxin $B_1$ ($AFB_1$); Aflatoxin $B_2$ ($AFB_2$); Aflatoxin $G_1$ ($AFG_1$); Aflatoxin $G_2$ ($AFG_2$).*

*b: Chemical structure of Aflatoxin hydroxylated metabolites. Aflatoxin $M_1$ ($AFM_1$); Aflatoxin $M_2$ ($AFM_2$); Aflatoxin $P_1$ ($AFP_1$); Aflatoxicol (AFL).*

## Materials and Methods

### Sampling

A total of 36 major commercially available brands of cheeses were obtained from different supermarkets in Mexico City in 2018. One hundred gram samples of 12 fresh and 24 mature cheeses of different brands were included in the analysis of AF (Table 1).

| N° | Type | Cheese name | Brand |
|---|---|---|---|
| 1 | Fresh | Fresh cheese (Panela) | Caperucita |
| 2 | | Fresh cheese (Panela) | FUD |
| 3 | | Fresh cheese (Panela) | Alpino |
| 4 | | Panela with wormseed | Wallander |
| 5 | | Cream cheese | Great Value |
| 6 | | Cream | Philadelphia |
| 7 | Fresh | Thread cheese (Asadero) | San Jacinto |
| 8 | | Fresh Barr | La Villita |
| 9 | | Mozzarella | Esmeralda |
| 10 | | American | Lala |
| 11 | | Melted American | La Villita |
| 12 | | American imitation | Aurrera |

| | | | |
|---|---|---|---|
| 13 | Mature | Sheep Manchego | Casa del Campo |
| 14 | | Dry cheese (Cotija) | Esmeralda |
| 15 | | Dry cheese (Cotija) grated | Del Parral |
| 16 | | Edam | Camp. Holandés |
| 17 | | Manchego | Ciervo |
| 18 | | Mented Manchego | La Villita |
| 19 | Mature | Manchego imitation | Aurrera |
| 20 | | Manchego | Noche Buena |
| 21 | | Manchego | La Villita |
| 22 | | Cheddar | Arla |
| 23 | | Cheddar with smoked chili (chipotle) | Wallander |
| 24 | | Cheddar | Wallander |
| 25 | Mature | Havarti | Arla |
| 26 | | Gouda | Holland Kroon |
| 27 | | Feta with herbs | Arla |
| 28 | | Camembert (French) | Maubert |
| 29 | | Camembert cheese | Carol |
| 30 | | Camembert cheese | Rosenborg |
| 31 | Mature | Camembert cheese | Reny Picot |
| 32 | | Blue cheese | Jans Kerk |
| 33 | | Blue cheese | D Áffinois |
| 34 | | Blue cheese | Gallo Dorado |
| 35 | | Blue cheese | Pico de Europa |
| 36 | | Blue cheese | Danablu |

***Table 1:*** *Mature and fresh cheese samples from Mexico City (ng $g^{-1}$).*

All samples were transported to the laboratory and processed immediately.

## Validation of the method

Validation was performed by applying the AOAC (2006) method [19] considering the parameters of linearity, limits of detection (LOD) and quantification (LOQ), percentage of recovery and selectivity [20]. To calculate the recovery percentage from each cheese sample, 15g was taken, different concentrations (5, 20 and 40 ng $mL^{-1}$) were prepared, and the chemical extraction method [19] was used to analyze the spiked and clean samples, to validate the method.

Eight concentrations of 1000 ng of each one of the following 8 AF (Sigma-Aldrich, St. Louis MO, USA) standards were prepared as stock solutions: $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$, $AFM_1$, $AFM_2$, $AFP_1$ and AFL. A spectrophotometer (Genesys 10 UV Thermo Electro Corporation UV/Vis Madison WI, USA) was used to measure the AF absorbance, and the concentration was calculated with the molecular weight and the extinction coefficient per AF in agreement with the physicochemical properties of each AF [7].

The AF calibration curves were calculated (Supplementary figure 2a and 2b) with 12 dilutions of the AF stock concentration, which were later dried at 40°C in an oven (Novatech BTC-9100 Houston, Texas, USA) and derivatized [21,22]. The concentrations of the different standards and the derivatized samples were analyzed by HPLC in triplicate, and the coefficient of determination ($R^2$) was calculated in Excel.

## Chemical extraction method

Subsamples (25g) from the 36 cheese samples were chemically extracted to purify and concentrate AFs.

The AFs and their hydroxylated metabolites were chemically analyzed according to the R-Biopharm user's guide (2012) [23] method with immunoaffinity columns (Easi-Extract total Aflatoxin, R-Biopharm Rhone Ltd., Glasgow, Scotland, UK).

The 36 cheese eluates were later dried at 40°C in oven, derivatized, and subjected to HPLC in triplicate to identify and quantify AFs.

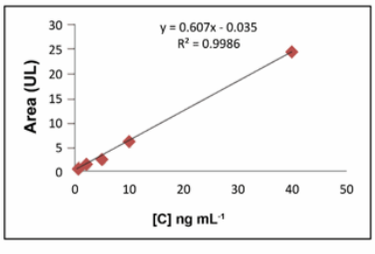
## HPLC quantification

The HPLC quantification method was followed [19]. The chromatographic system was an Agilent 1200 Series HPLC (Agilent Technologies, Inc. Santa Clara, CA, USA) equipped with a fluorescence detector (G1310A Series DE62957044, Agilent Technologies, Inc.). The excitation wavelength was set at 360 nm, and the maximum emission was 425 (for $AFG_1$ and $AFG_2$) or 450 nm (for the rest of the AFs). The chromatographic column was a VDS Optical VDSpher 100 C18-E (5 µm 250 x 4.6 mm), and it was maintained at room temperature (22°C). The degassed mobile phase was water:ACN:methanol (65:15:20 v/v/v) at a flow rate of 1.0 mL/min.

The concentration and absorbance values of the 12 dilutions of the AF standards were prepared to obtain the calibration curves (Supplementary figure 2a and 2b).

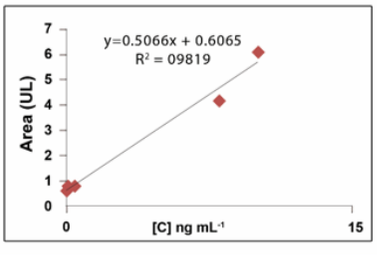
**AFB1**

| [C] (ng mL$^{-1}$) | Area (UL) | RT (min) |
|---|---|---|
| 0.5 | 0.341 | 8.849 |
| 1 | 0.993 | 8.695 |
| 2 | 1.295 | 7.877 |
| 5 | 2.357 | 7.638 |
| 10 | 5.995 | 7.347 |
| 40 | 24.317 | 7.085 |



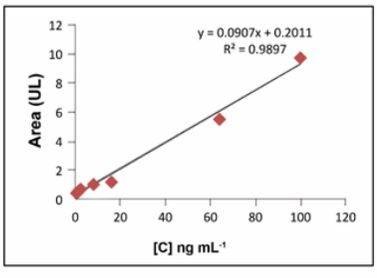

**AFB2**

| [C] (ng mL$^{-1}$) | Area (UL) | RT (min) |
|---|---|---|
| 0.05 | 0.63 | 17.452 |
| 0.1 | 0.8 | 18.141 |
| 0.5 | 0.82 | 18.93 |
| 8 | 4.15 | 20.062 |
| 10 | 6.08 | 20.228 |




**AFG1**

| [C] (ng mL$^{-1}$) | Area (UL) | RT (min) |
|---|---|---|
| 0.5 | 0.44 | 8.473 |
| 1 | 0.46 | 7.681 |
| 2 | 0.58 | 9.541 |
| 8 | 0.96 | 9.003 |
| 16 | 1.19 | 9.166 |
| 64 | 5.46 | 9.101 |




**AFG2**

| [C] (ng mL$^{-1}$) | Area (UL) | RT (min) |
|---|---|---|
| 0.5 | 0.20 | 14.177 |
| 1 | 1.05 | 11.215 |
| 2 | 1.57 | 12.062 |
| 4 | 5.91 | 12.002 |
| 10 | 12.78 | 14.507 |
| 16 | 17.47 | 14.513 |
| 32 | 35.12 | 14.476 |
| 64 | 91.13 | 14.492 |
| 100 | 159.66 | 14.464 |

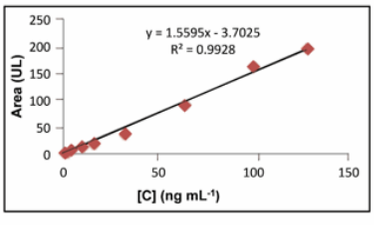



***Figure S2a***

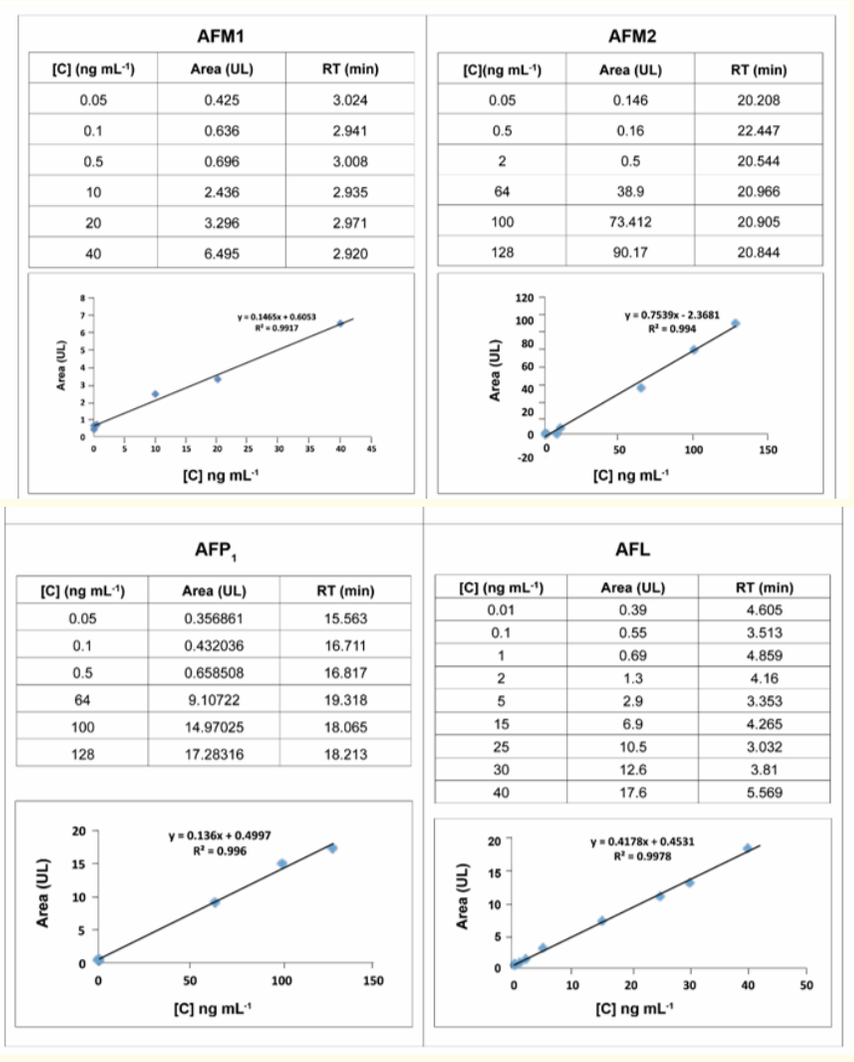



**Figure S2b**

***Figure S2****: a) Calibration curves of the 4 basic aflatoxins. b) Calibration curves of 4 hydroxylated metabolites of aflatoxins. Area UL = Area luminescence units; [C] (ng mL$^{-1}$) = Concentration (ng mL$^{-1}$); RT (min)= Retention time (minutes). $AFB_1$ = Aflatoxin $B_1$; $AFB_2$ = Aflatoxin $B_2$; $AFG_1$ = Aflatoxin $G_1$; $AFG_2$ = Aflatoxin $G_2$. $AFM_1$ = Aflatoxin $M_1$; $AFM_2$ = Aflatoxin $M_2$; $AFP_1$ = Aflatoxin $P_1$; AFL = Aflatoxicol.*

## Statistical methods

Kruskal-Willis analysis of the different AFs were obtained to find significant differences, and Wilcoxon rank test by pairs of cheeses on that AF.

## Results

### Validation

All the validation parameters (LOD, LOQ, $R^2$, Retention time, Percentage of recovery, and Sensitivity) were in optimal conditions (Table 2).

| Aflatoxin | LOD (ng $g^{-1}$) | Linearity (calibration curves) | | Recovery percentage |
|---|---|---|---|---|
| | | Retention time (min) | $R^2$ | |
| AFB1 | 0.01 | 7.085-8.849 | 0.9986 | 97% |
| AFB2 | 0.02 | 17.452-20.228 | 0.9819 | 95% |
| AFG1 | 0.05 | 7.681-9.541 | 0.9897 | 93% |
| AFG2 | 0.05 | 11.215-14.513 | 0.9928 | 96% |
| AFM1 | 0.01 | 6.44-6.59 | 0.9917 | 95% |
| AFM2 | 0.05 | 20.208-22.447 | 0.9940 | 97% |
| AFP1 | 0.05 | 15.563-19.318 | 0.9960 | 95% |
| AFL | 0.01 | 3.032-5.569 | 0.9978 | 98% |

***Table 2:*** *Validation parameters of aflatoxins and their hydroxylated metabolites.*

*LOD = Limit of Detection; $R^2$= Coefficient of Determination, min = Minutes.*

## Quantification of aflatoxins

AFL was the prevalent mycotoxin in 26 (72%) positive samples. Manchego, cream cheese and Gouda had no contaminants, and only six positive samples (17%) did not surpass the limit of 4 ng $g^{-1}$ of total aflatoxins permitted by international legislation (Table 3).

| N° | Cheese Name | Aflatoxins (μg $kg^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Basic AF | | | | AF hydroxylated metabolites | | | | |
| | | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ | $AFM_1$ | $AFM_2$ | $AFP_1$ | AFL | AFt |
| | **Fresh** | | | | | | | | | |
| 1 | Fresh cheese (Panela) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 45 |
| 2 | Fresh cheese (Panela) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 3 | Fresh cheese (Panela) | 0 | 0 | 0 | 0 | 105 | 0 | 0 | 0 | 105 |
| 4 | Panela with wormseed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 55 |
| 5 | Cream cheese | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | Cream | 0 | 0 | 0 | 0 | 125 | 0 | 0 | 1 | 126 |
| 7 | Thread cheese (Asadero) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 91 | 91 |
| 8 | Fresh Barr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| 9 | Mozzarella | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| 10 | American | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| 11 | American melted | 35 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 51 |
| 12 | American imitation | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 |
| | **Mature** | | | | | | | | | |
| 13 | Sheep Manchego | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | Dry cheese (Cotija) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 174 | 174 |
| 15 | Cotija grated | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 74 | 79 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 16 | Edam | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| 17 | Manchego | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 18 | Manchego melted | 0 | 0 | 0 | 0 | 106 | 0 | 0 | 0 | 106 |
| 19 | Manchego imitation | 0 | 0 | 0 | 0 | 132 | 0 | 0 | 0 | 132 |
| 20 | Manchego | 0 | 0 | 0 | 0 | 104 | 0 | 0 | 7 | 111 |
| 21 | Manchego | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 68 |
| 22 | Cheddar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 39 | 39 |
| 23 | Cheddar ¥ | 0 | 0 | 0 | 0 | 99 | 0 | 0 | 0 | 99 |
| 24 | Cheddar | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 49 | 92 |
| 25 | Havarti | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| 26 | Gouda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | Feta with herbs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 45 |
| 28 | Camembert | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| 29 | Camembert cheese | 0 | 0 | 0 | 0 | 107 | 0 | 0 | 1 | 108 |
| 30 | Camembert cheese | 0 | 0 | 0 | 0 | 108 | 0 | 0 | 40 | 148 |
| 31 | Camembert cheese | 0 | 0 | 0 | 0 | 115 | 0 | 0 | 7 | 123 |
| 32 | Blue cheese | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 29 |
| 33 | Blue cheese | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| 34 | Blue cheese | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 87 | 87 |
| 35 | Blue cheese | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 80 |
| 36 | Blue cheese | 0 | 0 | 0 | 0 | 195 | 0 | 0 | 65 | 260 |
| | Average | 3 | 0 | 0.4 | 0 | 35 | 0 | 0 | 26 | 64 |
| | Frequency | 4/36 | 0 | 1/36 | 0 | 11/36 | 0 | 0 | 26/36 | 33/36 |

***Table 3:*** *Aflatoxins and their hydroxylated metabolite concentrations in cheeses bought in Mexico City.*
*¥ Cheddar with smoked chili (chipotle).*

Four samples (11%) contained standard AFs, and $AFB_1$ was the most prevalent AF. Nevertheless, in 30 (83%) cheese samples, hydroxylated metabolites of AFs were detected. $AFB_2$, $AFG_2$, $AFM_2$ and $AFP_1$ were not detected.

Thirty-three (92%) cheese samples had at least one AF or hydroxylated metabolite (Figure 1). The morphology of *P. roqueforti* is shown (Figure 2).

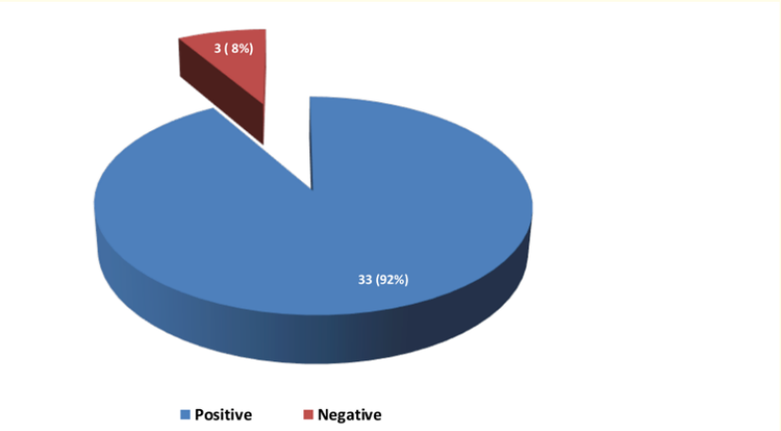
There was no difference in AF between fresh and mature cheeses. AFs ($AFB_1$, $AFG_1$, $AFM_1$ and AFL) are a health risk.

## Statistical analysis

We performed Kruskal-Willis analysis of the different AFs and found significant differences in $AFB_1$, $AFM_1$ and AFL (P values < 0.0001). We performed Wilcoxon rank test to analyze these AFs in pairs of cheeses.

On the other hand, the three fresh American cheeses (samples 10, 11 and 12) had $AFB_1$, which is the most toxic carcinogen among all AFs, and the last two samples contained amounts > 20 ng $g^{-1}$, shown to cause mutations [24].

Finally, 26/36 (= 72%) of the cheeses contained AFL, which can interconvert to $AFB_1$ and is a health risk.




| Contamination Range (ng g$^{-1}$) | Number and percentage of contaminated samples |
|---|---|
| ≤ 0.005* | 3 (8%) |
| > 0.005 ** | 33 (92%) |

*Non detectable; **Aflatoxin positive samples.

***Figure 1:*** *Occurrence of aflatoxins in cheese samples (ng g$^{-1}$) in Mexico city.*

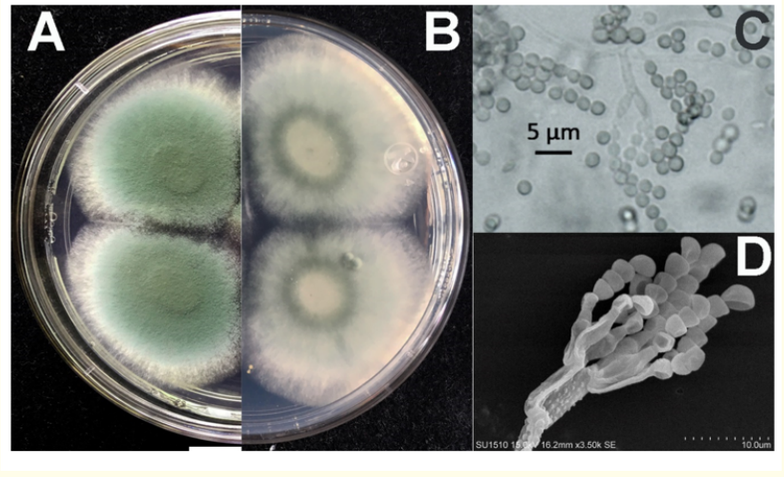



***Figure 2****: Representative morphology of P. roqueforti isolates from cheese samples traded in Mexico, showing light greenish gray colony color, velvety texture, regular and thick colony margins (A and B), ovoid conidia (C), and conidiophore stipe roughness (C and D).*

## Discussion and Conclusion

It is important to consider AFL levels in cheese because this hydroxylated metabolite interconverts with $AFB_1$ to become a dangerous carcinogen that was present in most of the analyzed cheeses (33/36), and most of the $AFB_1$ was metabolized to AFL [25].

AFL is produced by *Aspergillus flavus, A. parasiticus* and other *A. flavus* (non-aflatoxin-producing) by a reduction of the 1-keto group of $AFB_1$ [26].

To protect from $AFB_1$, the liver of mammals produces hydroxylated metabolites, such as AFL, as a detoxification mechanism. The formation of AFL is not truly a significant detoxification mechanism because it is still a potent carcinogen [27] that has approximately 70% of the mutagenicity of $AFB_1$.

AFL is present in two chemical structures, A (Ro) and B, both of which are produced from the biological European of $AFB_1$ mainly by *Tetrahymena pyriformis*, *Dactylium dendroides* and *Rhizopus* spp., among other microorganisms [28]. AFL A, used in the present study, was 18 times less toxic than $AFB_1$ in a duckling biliary hyperplasia assay. AFL B has an unknown biological activity. AFL is the major metabolite of $AFB_1$ in many plants and animals [28]. It has been detected in different matrixes such as milk, fermented dairy products [29], cereals, nuts, eggs, blood the human brain [30], sera and liver of humans with kwashiorkor and marasmic kwashiorkor [31], the muscle of broiler chickens fed a contaminated diet, and poultry fed chronic low doses of mycotoxins, with liver having the highest levels. In cattle, $AFB_1$, $AFM_1$ and AFL accumulate in the tissues and are excreted in the urine of calves. AFL–DNA adducts produced *in vivo* were identical to those produced by $AFB_1$, with similar molecular dosimetry responses and toxicity to the target organ. The electronic properties of AFL are calculated as similar to AFB1 and this may be an explanation of similar carcinogenicity and toxicity of these compounds [32]. AFL induced hepatocellular carcinomas in rats and fish, with lower tumor incidence than $AFB_1$ [33,34].

There is an interconversion of $AFB_1$ and AFL, mediated by intracellular enzymes, in rat blood, in guinea pigs, in sharks, which reconvert 30% of AFL to $AFB_1$, and in cultured human epidermal cells [29]. AFL converts to $AFB_1$, the most carcinogenic and toxic AF, during its formation in several fungi (*Aspergillus niger, Eurotium herbariorum, Rhizopus* spp. and non-aflatoxin-producing *A. flavus*). AFL is readily oxidized back to $AFB_1$, so it can serve as a 'reservoir' for $AFB_1$ *in vivo*, prolonging its effective lifetime in the body.

If pH has a role in the interconversion $AFB_1$-AFL, it could act in the normal digestion of milk in humans, in which pepsin lowers the pH. Isomerization of AFL to $AFB_1$ was observed in culture media with low culture pH [29]. In molecular dosimetry, DNA adduction and hepatocarcinogenicity in rainbow trout [35,36]; the tumorigenic potencies were as follows: $AFB_1$, 1.00; AFL, 0.936; $AFM_1$, 0.086; and $AFLM_1$, 0.041. AFL can be converted into $AFM_1$ and is a more potent toxin than $AFM_1$.

Monitoring AFL in cheese in Mexico is the first step to support the establishment of legislation that could diminish the risk to consumers and protect commercial activities. In cheese, the microbiota plays a key role in contributing to flavor, aroma, texture, and appearance. This complex microbial community develops throughout the ripening period and may be divided into two large groups: starters and secondary organisms.

Risk assessment parameters for $AFB_1$, AFL and $AFM_1$ were compared and the virtually safe dose for AFL was 1.7 times higher than that for $AFB_1$ [33,34].

Hepatocellular carcinoma incidence in rats and fish treated with AFL was lower than that in animals treated with $AFB_1$ at the same dose.

AFL in cheese might still be a health hazard, taking into account the amount of cheese consumed daily worldwide, but AFL is not legislated, and risk analysis is difficult if the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has no tolerance limits or parameters.

$AFM_1$ is an abundant AF in cheese, and the risk increases when AFL contamination in cheese is added [29]. Some factors, such as weather (rain or dry periods and temperature) during crop harvest, could contribute to fungal infection of feed cereals and AF production; grain storage time could increase the problem [29].

Except for $AFB_1$, the other AFs are not considered toxicologically important in the legislation of many countries. On the other hand, European Union legislation agreed that all kinds of food ought to be free of AFs [4].

The solution is not easy, and there are some remedies, such as Oltipraz from cruciferous vegetables, that reduce $AFB_1$ adduct biomarkers and inhibit $AFM_1$ production by bovine hepatocytes. In addition, it is necessary to balance the availability of cheese in relation to the

health risk, not only for cancer but also for other symptoms such as immune suppression, hepatitis, cirrhosis, Reye's syndrome, etc. This fact makes AF regulation very problematic.

AFs are recurrent and sometimes unavoidable contaminants of cheese because they are present in the cereals and oilseeds used to feed cattle, and AFs have thermal stability and inhibit pasteurization and ultrapasteurization as good control methods. Microbial degradation of aflatoxin $B_1$ and AFL is a promising control possibility for future methods. Other $AFB_1$ detoxification processes happened, by the caterpillar *Trichoplusia ni*, even with *Fusarium* that is a mycotoxigenic fungi [35,36]. The chemical control methods were the fastest that still retained high detoxification efficacy. The best control strategy is to keep raw materials and feed under obligatory AF regulation. The same cheese sample sometimes had several AFs and/or AFL. Health risk for humans is due to the frequency of consumption, and the lack of care in the sources of exposure as the cattle quality of industrialized feed, because a cow that eats grass produces AF clean milk.

This study showed the presence of carcinogenic AFL in 92% of cheeses in Mexico with a contamination range of 1.1 to 174.3 ng $g^{-1}$ and an average AFL contamination in cheese of 26.5 ng $g^{-1}$ or > 20 ng $g^{-1}$ in 14 of 36 samples ($p < 0.0001$). The concentration of AFL found in cheese could be a health risk if it accumulates in DNA over time and also due to the interconversion of AFL to $AFB_1$, that increases the danger. Our results highlight the urgent need to implement efficient quality control monitoring to fulfill the expected quality requirements and to meet Mexican sanitary regulations and specifications for imported dairy.

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## Conflicts of Interest Statement

All authors disclose any financial conflict of interest in the results or interpretation of this manuscript.

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