

## Aflatoxins, Zearalenone, Fumonisin and Deoxynivalenol Multiple Contamination in Raw Maize (*Zea mays* L.), for Human Consumption

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### Abstract

The aim of this study was to investigate the multi-mycotoxin contamination in maize. A total of 31 raw maize samples were collected - ten from Mexico City and 21 from eight municipalities from the State of Puebla. The samples were prepared, identified and analyzed in the Toxicology Laboratory at UAM-X, using the Enzyme-Linked Immunosorbent Assay (ELISA-indirect) and High Liquid Chromatography coupled to a fluorescence detector (HPLC-FD). A two factor ANOVA was performed (FUM and DON) and three levels (A, B, N) using Minitab, 2018 Software. The present study detected multi-mycotoxin contamination by Zearalenone (ZEA), Fumonisin (FUM), Deoxynivalenol (DON) and aflatoxins (AFL) in maize samples from Puebla and Mexico City. The interaction between DON and FUM was statistically significant ( $P < 0.05$ ). It is concluded the need to analyze the fusariotoxin and aflatoxin in foods for children due to their genotoxicity and carcinogenicity.

**Keywords:** Multiple Contamination; Fusariotoxins; Aflatoxins; Raw Maize

### Introduction

Maize is the most important crop in Mexico, with a production of 23 million tons per year and high consumption [1]. The central states of the country, are those with the largest planted area, although with the lowest yields per hectare, due to the lack of technology in water and crop diseases that affect crop yield and the producer's economy [1,2].

*Fusarium* spp is a saprophyte filamentous fungus of agricultural soil that adapts to temperate and humid climates and produces 139 fusariotoxins [3]. *Fusarium verticillioides* Sheld and *F. proliferatum* produces Fumonisin (FUM), *Fusarium culmorum* W.G., Deoxynivalenol (DON) and Zearalenone (ZEA); *Fusarium graminearum* Sch., *F. cerealis*, synthesizes more than 17 mycotoxins, including Zearalenone (ZEA) and Deoxynivalenol (DON) [4,5].

*Aspergillus flavus* is a fungus that produces aflatoxins [6] and grows in warm weathers. It has been identified four aflatoxins forms (AFB1, AFB2, AFB3, AFB4) at least, but the AFB1 is the most dangerous, due to damage in nucleic acids and nucleoproteins with cytotoxic and genotoxic effects [3].

Once the fungus is installed and the weather is specific for mycotoxins synthesis, the mycotoxins remain stable even under industrial processes [7]; therefore, they can be detected in flour, breakfast cereals and bread [8,9]. It is crucial to ensure the raw material's quality for human consumption manufacture principally in a vulnerable population like children [5,10].

Zearalenone (ZEA), is a hepatotoxin, with potential estrogenic and androgenic activity. It has been identified as responsible for cancer in genital organs in animals and early development of breasts in girls and genitals in boys [11]. It induces oxidative stress and the generation of free radicals, leading to a genotoxic effect, which results in the induction of adducts, micronuclei and chromosomal aberrations [2,13]. The maximum permissible limit for Zearalenone is 20  $\mu\text{g kg}^{-1}$  for baby food [14] and 100  $\mu\text{g kg}^{-1}$  for food in general, according to the FDA -USDA of the United States.

Fumonisin (FUM) are a group with at least 15 compounds, but FB1 is the most significant. It has been established their capability of inhibiting ceramide synthase, blocking the synthesis of the sphingolipids, causing growth damage, differentiation and cellular death [15,16]. FUM intake was related to esophageal cancer in humans [17,18]. The MPL established is 200  $\mu\text{g kg}^{-1}$  [14] for baby food and 2000  $\mu\text{g kg}^{-1}$  for food in general.

Deoxynivalenol (DON), can inhibit protein synthesis and decrease the immune system activity, so the exposure has been associated with alterations in the intestinal immune, endocrine and nervous systems [19] as well as with hepatitis and cancer [20]. The MPL in European countries is 50  $\mu\text{g kg}^{-1}$  [14] and 1000  $\mu\text{g kg}^{-1}$  for food in general [21].

The Enzyme-Linked Immunosorbent Assay or ELISA is a rapid analysis technique validated by the United States Federal Grain Inspection System (FGIS-USDA, 2017) and considered very useful as a routine analysis for the mycotoxins detection cereal grains [9]. Its principle is the specific binding of an antigen (mycotoxin) and an antibody, by enzymes; however, it is convenient to check the results with some other method of greater sensitivity and specificity such as liquid chromatography or mass-coupled HPLC [22,23].

## Material and Methods

Thirty-one maize samples were collected; 10 from Mexico City (four from Xochimilco and 6 from Tlahuac); twenty-one samples from eight municipalities of Puebla. From the 31 collected samples of the State of Puebla, twenty-one were analyzed for zearalenone (ZEA), twelve for fumonisin (FUM), fifteen for deoxynivalenol (DON) and twelve for aflatoxins (AFL). From the samples of Mexico City, eight were analyzed for ZEA, nine for FUM, ten for DON and six for AFL.

### Sample preparation

500g of each sample was transported to the Toxicology Laboratory of UAM-X where they were cleaned, weighed and identified; consequently, samples were divided into sub-samples of 100g each, ground in an electric mill and sieved to a 1 mm particle size.

### Fusariotoxins analysis

Indirect-ELISA assay was performed to determine qualitatively and quantitatively each mycotoxin, according to the specifications of a commercial kit for cereal grains from 5g of each sample.

Ridascreen ELISA kit (R-Biopharm, Germany) was used for zearalenone, deoxynivalenol and fumonisin. The kit for zearalenone with a detection limit of 17  $\mu\text{g kg}^{-1}$ ; DON and FUM of 25  $\mu\text{g kg}^{-1}$ . The extraction procedure for ZEA was in methanol: water (70:30 v/v). the extract was diluted to a volume of 1:1 with distilled  $\text{H}_2\text{O}$ . Three washes were performed on the plate with 250  $\mu\text{l}$  of distilled  $\text{H}_2\text{O}$ . In case of DON, 100 mL of distilled  $\text{H}_2\text{O}$  was added to the sample. Filtering and the addition of the corresponding enzyme and antibody reagents. The three washes were performed with 250  $\mu\text{l}$  of wash buffer (PBS-Tween). For FUM 25 mL of a solution of methanol: water (70:30 v/v) was added. Subsequently, the extract was diluted to a volume of 1:14 with distilled  $\text{H}_2\text{O}$ . All the mycotoxins were photometrically determined

at a wavelength of 450 nm, in a Biotek ELISA plate reader model ELX800 and quantification with the commercial software RIDA SOFT Win. The results were expressed in  $\mu\text{g kg}^{-1}$ .

**HPLC-FD aflatoxins detection**

For the aflatoxins analysis, it was used a Varian ProStar 325 HPLC, coupled with a fluorescence detector (HPLC-FD). with a nucleosil column C18, 125 x 4.6 mm, isocratic pump model PS230, and a software Galaxie version 1.9 SP2. The chromatographic conditions were 1 mL/min pump flow, 5  $\mu\text{L}$  injection volume, excitation 360 nm and emission 440 nm. The mobile phase acetonitrile-methanol-water (2:2:6). The acetonitrile-water 9:1 and a derivatizing solution water-trifluoroacetic acid-acetic acid 7:2:1. The limit of detection was 0.06  $\mu\text{g kg}^{-1}$ .

**Statistical analysis**

A two-factor ANOVA (FUM and DON) was performed with three levels (concentration) A, B, N, using the Minitab software, 2018.

**Results and Discussion**

The present study detected a multi-mycotoxin contamination by Zearalenone (ZEA), Fumonisin (FUM), Deoxynivalenol (DON) and Aflatoxins (AFL) in maize samples from Puebla and Mexico City. The ZEA levels in maize from Puebla was 108.42 ppb, and 172.49 ppb for Mexico City samples. FUM levels of  $341.14 \pm 148.83 \mu\text{g kg}^{-1}$  and  $375.67 \pm 148.9 \mu\text{g kg}^{-1}$ . DON levels were of  $381.53 \pm 187.77 \mu\text{g kg}^{-1}$  and  $588.2 \pm 497.41 \mu\text{g kg}^{-1}$ . The aflatoxin mean levels were 3.62 ppb and 6.89 ppb respectively. Aflatoxins were present too in the lowest levels, however, in samples from Mexico City above regulatory limits of 5  $\mu\text{g kg}^{-1}$  (Table 1 and 2).

	ZEA	FUM	DON	AFLA
Positive samples	21	12	15	16
Frequency	100%	57%	72%	57%
Range	52.13 - 276.87	90 - 580	220 - 812	2 - 7.24
Mean	$108.42 \pm 66.02$	$341 \pm 148.83$	$381.53 \pm 187$	3.62

**Table 1:** Fusariotoxins frequency and levels ( $\mu\text{g kg}^{-1}$ ) in maize samples from Puebla.

	ZEA	FUM	DON	AFL
Positives samples	8	9	10	6
Frequency	8/10	9/10	10/10	6/10
Range	31.51 - 329.9	220 - 551	22 - 1710	2.0 - 7.8
Mean	$172.49 \pm 102.60$	$375.67 \pm 148.9$	$588.2 \pm 497$	6.89

**Table 2:** Zearalenone, Fumonisin, Deoxynivalenol frequency and contamination levels in maize samples from Mexico City.

In this study a multi-mycotoxin contamination in maize from two states of Mexico, with a temperate weather was observed. The contamination might have been because of climate change, since there are changes in precipitation patterns and atmospheric CO<sub>2</sub> levels. Fungi and insects are being displaced by more aggressive ones, such is the case of *Fusarium verticillioides*, and *F. graminearum* which can synthesize fumonisin, deoxynivalenol and zearalenone; however the levels of fumonisin and deoxynivalenol detected, were within the USA regulation. The detected levels of Zearalenone in maize are worrying since it is an estrogenic mycotoxin that acts on the reproductive system and has been related to early physical changes in girls breasts who had consumed contaminated food.

## Conclusion

Multi-contamination of mycotoxins in raw maize from the State of Puebla and Mexico City was demonstrated. Nevertheless, all fusario-toxins were within the FDA-USDA regulation of the United States for human consumption food. The inspection and regulation of fusario-toxins in maize-based food should be considered.

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