# Occurrence of Fumonisin $B_1$ in Maize Grown in Costa Rica

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*Fusarium moniliforme* is one of the most frequent fungal pathogens found in corn. Samples of corn grown in three different regions of Costa Rica were collected and analyzed for the presence of fumonisins, metabolites produced by *F. moniliforme*. Results indicate that fumonisin $B_1$ was present in 89% of the samples. Maize grown in Costa Rica is therefore contaminated with fumonisin $B_1$, and contamination depends on geographical region and period of collection. Microbiological analysis revealed the presence of *F. moniliforme*-type isolates in 86% of the maize samples. Average levels of fumonisin $B_1$ per region—Brunca, Huetar Norte, and Huetar Atlantica—were 2.50, 3.58, and 1.81 ppm, respectively. Mean fumonisin $B_1$ values were 2.13 ppm with a minimum of 0.00 ppm and a maximum of 6.32 ppm. Maize from the Huetar Atlantica region showed significantly ($a = 0.05$) higher levels of *F. moniliforme*-type isolates than maize from the Brunca and Huetar Norte regions.

**Keywords:** *Fumonisin; maize; Costa Rica*

## INTRODUCTION

Fumonisins are defined as fungal metabolites produced by *Fusarium moniliforme* which is nearly ubiquitous in maize. Because of its association with toxicology problems in animals and humans, and consequent economical losses in maize production, this fungus has been the focus of food toxicologists (Summer, 1968; Marijanovic et al., 1991; Bacon et al., 1992; Plattner et al., 1992; Zummo and Scott, 1992). Among fumonisins, fumonisin $B_1$ ($FB_1$) seems to be the major fumonisin produced naturally by *F. moniliforme* (Ross et al., 1992; Plattner et al., 1992). Fumonisins have been shown to be heat stable (Alberts et al., 1990) as well as widely distributed in regions with warm and humid tropical and subtropical conditions (Bacon and Williamson, 1992).

Costa Rica is an agricultural country where grain production, especially maize, is an important part of the economic activity. The occurrence of fumonisin in maize grown in Costa Rica for human consumption has not been evaluated at the present time. This is necessary because maize is an important source of income for small local farmers and is used as the principal component in tortillas, biscuits, chips, and other popular food items. A previous study showed that maize grown in the region was contaminated with aflatoxins (Viquez et al., 1994). The objective of this research was to determine the presence of *F. moniliforme*-type isolates and fumonisins in maize samples grown at three different locations in Costa Rica. This is the first study of the natural occurrence of fumonisin in maize produced in the region.

## MATERIALS AND METHODS

**Maize Samples.** Samples of maize of the 1992–1993 crop were obtained from three different regions in Costa Rica: Brunca, Huetar Atlantica, and Huetar Norte. For the purpose of this study, two handling processes [farm harvesting and National Production Council (CNP) managerial], three agencies per region, and three periods of collection (2 months each) were used as sampling criteria. Periods were named as early (August–September), middle (October–November), and late (December–January).

The sampling at each agency for evaluation of farm harvesting and CNP conditions was carried out in three stages.

(1) A representative sample constituting 10% of the daily amount of corn purchased by each agency was collected. Weekly samples were collected at each agency to be representative. Approximately 5 kg samples were collected daily with a total weight of 25 kg at the end of each week.

(2) An 8 kg subsample which represented the purchased corn per agency per week was collected. These 8 kg samples were milled and reduced to an intermediate subsample size (mesh 40) using a rotary cascade sample divider. The third sample preparation was the reduction of the 8 kg to an intermediate subsample weighing approximately 2 kg.

Weekly subsamples (2 kg) were mixed with the collected samples of 3 weeks with a total weight of 6 kg. This subsample (6 kg) was reduced to a final subsample of 1 kg in weight which is a representative sample of each agency per region per period. These preparative steps were conducted using a hammermill.

This 1 kg sample of representative dried ground samples was collected by the CNP and shipped from Costa Rica to the United States (Alabama A&M University) by INCIENSA (National Institute of Research on Nutrition and Health). Each dried ground sample was stored in sealed plastic bags at 4 °C prior to analysis.

**Fumonisin Analysis.** The fumonisin content in the maize samples was determined using a modified version of the high-pressure liquid chromatography (HPLC) fumonisin method of Shephard et al. (1990). A derivatization method was used for fumonisin determination because the toxicant cannot absorb UV or visible light. Fumonisin was extracted from the maize samples (25 g) by shaking for 12 h with water and filtering through Whatman no. 4 filter paper. Prior to derivatization, samples were concentrated on a C-18 "sep-pak" column (Waters 20515). Columns were preconditioned with 2 mL of methanol followed by 5 mL of water. Ten milliliters of the filtered sample was then loaded on the sep-pak and washed with 10 mL of water. The sample was then eluted with 2 mL of methanol. Aliquots of samples (25 $\mu$L) were used for derivatization with *o*-phthaldialdehyde (OPA). Derivatives were prepared immediately prior to injection by the addition of OPA reagent (100 $\mu$L) and sample solution (25 $\mu$L). The OPA reagent was prepared by dissolving 40 $\mu$g of OPA in

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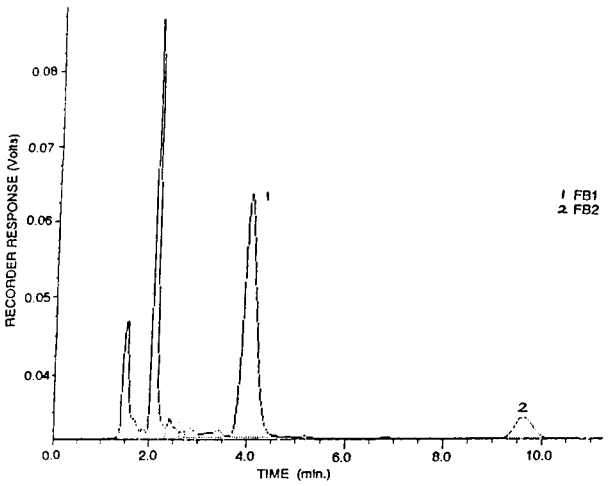


**Figure 1.** Typical chromatogram of fumonisin standards.

**Table 1. Levels (Parts per Million)[a] of Fumonisin $B_1$ Found in Maize Grown in Costa Rica for Human Consumption**

| | region and collection period | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $A_1$[b] | $A_2$ | $B_1$ | $B_2$ | $B_3$ | $C_1$ | $C_2$ | $C_3$ |
| | 2.25 | 1.41 | 2.32 | 2.20 | 6.56 | 0.00 | 1.98 | 1.73 |
| | 0.00 | 3.08 | 6.32 | 3.03 | 5.16 | 1.96 | 2.29 | 1.73 |
| | 1.79 | 3.07 | 6.16 | 2.14 | 4.23 | 3.70 | 0.55 | 1.73 |
| | 0.00 | 5.43 | 4.30 | 2.06 | 2.35 | 2.38 | 1.98 | 1.73 |
| | 1.42 | 1.41 | 2.32 | 2.20 | 3.51 | 0.00 | 2.29 | 1.73 |
| | 3.38 | 3.08 | 6.30 | 3.03 | 2.31 | 1.96 | 0.55 | 1.74 |
| | 1.18 | 5.43 | 6.20 | 2.14 | 1.79 | 3.70 | 1.98 | 1.70 |
| | 3.92 | 3.07 | 4.30 | 2.06 | 2.92 | 2.38 | 2.00 | 1.72 |
| mean | $1.74^{x}$ | $3.25^{y}$ | $4.78^{z}$ | $2.36^{x}$ | $3.60^{w}$ | $2.01^{x}$ | $1.70^{x}$ | $1.73^{x}$ |

[a] Values are means of eight replicates. Means with the same letter indicate no significant difference ($a = 0.05$). [b] A, Brunca; B, Huetar Norte; C, Huetar Atlantica; 1, early (August−September); 2, middle (October−November); and 3, late (December−January).

methanol (1 mL) and adding 5 mL of 0.1 M disodium tetraborate and 50 mL of 2-mercaptoethanol. Due to the instability of OPA derivatives, HPLC injection was made exactly 1 min after the sample solution was mixed with the OPA reagent.

HPLC conditions were as follows. The mobile phase was 75% HPLC grade methanol plus 25% 0.1 M $NaH_2PO_4$ adjusted to pH 3.3 with phosphoric acid at a flow rate of 2 mL/min. The column was a Waters C-18 "Novapack" column with a precolumn. Detection was by fluorescence (excitation = 335 nm, emission = 440 nm). Quantitation was by integration of peak areas using Waters *Baseline* software, with linear regression of peak areas against a standard curve of fumonisin $B_1$ and $B_2$ standards.

**Microbiological Analysis.** Counts of fungal populations present in the maize samples were performed by plating serial log dilutions on acidified potato dextrose agar (APDA) for *F. moniliforme*-type isolates as selective media. APDA was chosen on the basis of preliminary studies on different media, including Nash-Snyder, APDA, Czapek's, cornmeal agar, and others which showed acceptable growth of the fungus on APDA. Colonies were expressed as log colony-forming units (CFU's) per gram of maize.

## RESULTS AND DISCUSSION

A typical chromatogram obtained from a 10 $\mu$L aliquot of fumonisin standards is shown in Figure 1. Average levels of fumonisin $B_1$ per region—Brunca, Huetar Norte, and Huetar Atlantica—were 2.50, 3.58, and 1.81 ppm, respectively. Mean fumonisin $B_1$ values were 2.13 ppm with a minimum of 0.00 ppm and a maximum of 6.32 ppm (Table 1). Fumonisin $B_1$ was present in 89%

**Table 2. Fungal Counts at 4 Days in Acid PDA in Maize Grown in Costa Rica for Human Consumption**

| | *F. moniliforme*-type isolates (log CFU/g)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | region and collection phase | | | | | | | |
| | $A_1$[b] | $A_2$ | $B_1$ | $B_2$ | $B_3$ | $C_1$ | $C_2$ | $C_3$ |
| | 2.35 | 0.00 | 3.35 | 0.00 | 3.49 | 3.95 | 3.46 | 2.82 |
| | 3.43 | 2.95 | 0.00 | 3.30 | 4.00 | 3.05 | 4.51 | 2.82 |
| | 2.95 | 3.30 | 3.63 | 3.93 | 2.35 | 3.89 | 2.35 | 2.82 |
| | 2.95 | 3.82 | 2.95 | 3.90 | 3.12 | 3.35 | 3.46 | 2.82 |
| | 2.95 | 2.95 | 3.35 | 0.00 | 4.09 | 3.95 | 3.46 | 3.03 |
| | 2.35 | 3.30 | 0.00 | 3.30 | 3.89 | 3.05 | 4.51 | 3.03 |
| | 3.05 | 3.82 | 3.63 | 3.93 | 3.69 | 3.89 | 2.35 | 2.82 |
| | 3.58 | 0.00 | 2.95 | 3.90 | 0.00 | 3.35 | 3.31 | 3.03 |
| mean | 2.95 | $2.52^{x}$ | $2.48^{y}$ | $2.78^{z}$ | 3.08 | $3.56^{u,v,w}$ | $3.43^{u,v,w}$ | 2.90 |

[a] Values are means of eight replicates. Superscripts indicate a significant difference ($a = 0.05$) among samples. Thus, values for regions $A_2$, $C_1$, and $C_3$ and $B_1$, $C_1$, and $C_2$ were significantly different and so forth. No superscript means no differences among samples. [b] A, Brunca; B, Huetar Norte; C, Huetar Atlantica; 1, early (August−September); 2, middle (October−November); and 3, late (December−January).

of the maize samples. Fumonisin $B_2$ was not found in the maize samples.

ANOVA and Tukey's studentized methods for near separation tests indicate that there were significant differences ($a = 0.05$) in fumonisin levels as a function of geographical region and collection period. The collection period (within a region) made a difference for samples from the Brunca and Huetar Norte regions, but no significant differences in fumonisin levels were observed for the samples from the Huetar Atlantica region. Fumonisin levels in samples from the Brunca region were significantly ($a = 0.05$) different from the levels in samples from the other two regions, independent of the collection period. Differences in fumonisin levels were observed for the samples from the Huetar Norte and Huetar Atlantica regions only during the late (December−January) collection period. Thus, maize grown in Costa Rica is contaminated with fumonisin $B_1$ with levels varying within geographical region and maize collection period.

*F. moniliforme*-type isolates was present in 86% of the samples. Average values of *F. moniliforme*-type isolates per region were 2.74, 2.78, and 3.30 log CFU/g for Brunca, Huetar Norte, and Huetar Atlantica, respectively. Mean values were 2.96 log CFU/g with a minimum of 0.00 and a maximum of 4.51 (Table 2). Significant ($a = 0.05$) differences between samples from the Huetar Atlantica region and the other two regions were observed at all collection periods. No significant differences were observed between samples from the Brunca and Huetar Norte regions.

As a result, the difference in fungal infestation and fumonisin production between the regions and periods of collection could be due to differences in harvesting techniques, collection phase, storage conditions, source of *Fusarium liseola* section isolates in soil, and/or environmental conditions (temperature, relative humidity, and rainfall). The results could be related to the differences in agricultural practices in each region, where farmers used a different handling process before and during harvest. Also, the Huetar Atlantica region has the poorest agricultural practices among the regions.

The effect of rainfall and relative humidity on fumonisin levels showed a drastic variation during the harvesting season (March−August) which could have produced physiological stress on the crops.

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