 Publicación INCAP PCI/092

Journal of Food Protection, Vol. 62, No. 10, 1999, Pages 1218–1222

Research Note

Fumonisin B_1 and Hydrolyzed Fumonisin B_1 (AP_1) in Tortillas and Nixtamalized Corn (*Zea mays* L.) from Two Different Geographic Locations in Guatemala†

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MS 98-344: Received 23 December 1998/Accepted 22 June 1999

ABSTRACT

Fumonisin B_1 (FB_1) is a common contaminant of corn worldwide and is responsible for several diseases of animals. In the preparation of tortillas, corn is treated with lime (producing nixtamal) that when heated hydrolyzes at least a portion of the FB_1 to the aminopentol backbone (AP_1), another known toxin. This study analyzed the amounts of FB_1 and AP_1 in tortillas and nixtamal from two communities in the central highlands of Guatemala where corn is a major dietary staple (Santa Maria de Jesus, Sacatepequez, and Patzicia, Chimaltenango). The amounts of FB_1 and AP_1 in tortillas from Santa Maria de Jesus were, respectively, 0.85 ± 2.0 and 26.1 ± 38.5 µg/g dry weight (mean ± SD), and from Patzicia were 2.2 ± 3.6 and 5.7 ± 9.4 µg/g dry weight. Less than 6% of the tortillas from both locations contained ≥ 10 µg FB_1/g dry weight; whereas, 66% of the samples from Santa Maria de Jesus and 29% from Patzicia contained ≥ 10 µg AP_1/g dry weight. The highest amount of AP_1 (185 µg/g dry weight) was found in tortillas from Santa Maria de Jesus. The highest amounts of FB_1 were 6.5 and 11.6 µg/g dry weight in tortillas from Santa Maria de Jesus and Patzicia, respectively. The mean concentration of FB_1 in nixtamal was significantly higher in Santa Maria de Jesus compared to Patzicia. Surprisingly, AP_1 was not detected in any of the nixtamal samples. The human impact of exposure to these amounts of fumonisins is not known. However, based on findings with other animals, where corn is a dietary staple, long-term consumption of FB_1 and AP_1 (especially at ≥ 10 µg/g of the diet) may pose a risk to human health.

The fungus *Fusarium moniliforme* Sheldon occurs worldwide *(11)* on a variety of plant hosts and is common in corn that is used as animal feed and food consumed by humans. *F. moniliforme* and a number of additional species of *Fusarium* produce fumonisins *(2)* (the most prevalent of which is fumonisin B_1, FB_1) that appear to be responsible for diseases caused by consumption of corn contaminated with these fungi, corn screenings, and *F. moniliforme* culture material *(11)*. These include equine leucoencephalomalacia *(10)*, porcine pulmonary edema *(6)*, hepatotoxicity, nephrotoxicity, and hepatocarcinogenicity in rodents *(5)*. Less is known about the effects of fumonisins on humans, but consumption of *F. moniliforme* and fumonisins has been associated with esophageal cancer in the Transkei region of South Africa *(12, 22)* and Cixian and Linxian counties, China *(4)* and with liver cancer in Haimen, China *(24)*. In 1993, toxins derived from *F. moniliforme* were evaluated as possibly carcinogenic to humans by the International Agency for Research on Cancer *(1)*.

In the preparation of tortillas, corn is treated with a lime solution and heat (a process called nixtamalization) that can hydrolyze the tricarballylic acid side-chains from FB_1, leaving the aminopentol backbone (referred to as AP_1) *(8)*. FB_1 and AP_1 are inhibitors of ceramide synthase that is thought to be the major biochemical target for these compounds *(15)*, although AP_1 is less potent *(18)*. The hydrolysis of FB_1 does not appear to detoxify contaminated corn because AP_1 is also toxic to experimental animals *(7, 25)*.

FB_1 and AP_1 have been found in corn and foods prepared from corn in the United States and elsewhere. However, less is known about their prevalence in human foods in countries *(7)*, where corn is a substantial portion of the diet. In some regions of Central America, for example, as much as 70% of the total calories are provided by foods prepared from corn *(3)*. Therefore, the present study compared the amounts of FB_1 and AP_1 in tortillas, and, for comparison, the nixtamalized corn, from 50 different households from each of two different geographic locations in the central highlands of Guatemala: Santa Maria de Jesus, Sacatepequez, and Patzicia, Chimaltenango.

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† Mention of firm names or trade products does not imply the endorsement by the U.S. Department of Agriculture over firms or other commercial products.

MATERIALS AND METHODS

Samples. Tortillas and the unground nixtamal (see description below) were collected from 50 different households in Santa Maria de Jesus and Patzicia, Guatemala, during the period August to September of 1995. The nixtamalization process used by the Mesoamerican population *(3)* consists of boiling the corn kernels in a solution of calcium carbonate (created by adding lime) for at least 1 h and allowing them to soak. After 12 to 14 h, the lime water is discarded, and the corn is washed with water three to four additional times to remove the seed coat, tip cap, excess lime, and impurities in the seeds. The cooked wet grain is then ground with a meat grinder to produce masa or corn dough. In this study, the unground and dried nixtamalized corn was defined as nixtamal. Corn dough (masa) was not collected because it has a tendency to ferment in storage. The tortillas and nixtamal samples were then placed into ice chests and kept cold until they could be stored at −20°C. The frozen samples were packed in Styrofoam boxes with dry ice and frozen gel-packs, shipped to Miami, Fla. for import into the United States, and then forwarded to the U.S. Department of Agriculture laboratory in Athens, Ga. Some of the tortilla samples thawed during shipping and were not considered appropriate for inclusion in the study. Thus, analyses were conducted on tortilla samples from 38 households in Santa Maria de Jesus and 35 in Patzicia. Analyses were also conducted on nixtamal from 46 and 50 households in Santa Maria de Jesus and Patzicia, respectively. Corn samples were also collected and shipped to the United States, but these were found to be infested with insects and destroyed by U.S. Custom inspectors.

Extraction and analysis. The frozen tortillas and nixtamal were freeze dried, ground to pass through a 2-mm screen, and then duplicate samples weighing 5 to 10 g each were placed into 125-ml glass-stoppered Erlenmeyer flasks. The pH was adjusted to 4.5 with 0.1 N HCl, and acetonitrile–water (1:1, vol:vol) was added to each flask (25 ml) that was then sealed and placed on a platform shaker at 125 rpm. After 12 to 16 h of extraction at room temperature, each sample was filtered by gravity through Whatman no. 4 filter paper. A 2-ml aliquot of the extract was diluted with water to 8 ml and applied by syringe to an activated Waters C_{18} SepPak cartridge (the SepPak cartridge was activated by washing sequentially with 2 ml each of water, acetonitrile, and water). The SepPak was washed with 2 ml of water and 2 ml of 15% acetonitrile–water, and then the fumonisins were eluted with 2 ml of 70% acetonitrile–water.

Three 5-g samples of ground tortilla were spiked with 1 ml of acetonitrile–water (1:1) containing 33.4 μg fumonisin B_1 and 106 μg AP_1. The spiked samples were placed onto a heating block and heated at 50°C for 20 min under nitrogen gas to remove any liquids. The tortilla samples were then placed in a desiccator overnight to remove any further residual moisture. The samples were extracted and analyzed by high-performance liquid chromatography using the same procedures as the previous tortilla samples. The percent recovery of the FB_1 and the AP_1 that was spiked into the ground tortilla samples was determined to be 54.3 ± 0.1 and 52.1 ± 4.4, respectively.

The FB_1 and AP_1 were analyzed as their *o*-phthalaldehyde derivatives by high-performance liquid chromatography, and quantification of the amounts was accomplished by comparing the fluorescence with that of analytical standards, as described previously *(13)*. The analytical standard of FB_1 was prepared by the method of Meredith et al. *(14)*, and the purity (>94%) was determined by the procedure of Plattner and Branham *(19)*. The AP_1 was prepared by the method of Plattner and Branham *(19)* and mass spectral data were used to verify the purity.

TABLE 1. *Minimum (min), maximum (max), average (ave), and standard deviation (SD) for fumonisin B_1 and hydrolyzed fumonisin B_1 (AP_1) in tortillas from two different communities in the central highlands of Guatamala*[a]

	Tortillas (μg/g dry weight)			
	Min	Max	Ave	SD
Santa Marie de Jesus				
FB_1	<0.4	6.5	0.85	2.0
AP_1	<0.4	185.1	26.1	38.5
Patzicia				
FB_1	<0.4	11.6	2.2	3.6
AP_1	<0.4	31.7	5.7	9.4

[a] df = 72, means for FB_1 were not significant $P < 0.082$; df = 72, means for AP_1 significantly different at $P < 0.0003$.

Statistical analyses. Differences between groups were evaluated by analysis of variance using the general linear models procedure of SAS *(20)* on data that were normalized by taking the square root of each value. The *n* for the analyses of the tortillas was 73 (38 from Santa Maria de Jesus and 35 from Patzicia), and for nixtamal *n* was 96 (46 from Santa Maria de Jesus and 50 from Patzicia). Mean values are given ± standard deviation (SD) unless indicated otherwise.

RESULTS

Preparation of nixtamal and tortillas. The amounts of lime and water used in producing the nixtamal for this study varied from household to household and from community to community, but the overall procedure was basically the same as described in the Materials and Methods. In Santa Maria de Jesus, the average weight of corn used in the nixtamal process was 1.24 ± 0.47 kg/liter water, and the amount of lime added was 62.7 ± 51.4 g/liter of water, while in Patzicia the average weight of corn was 0.95 ± 0.68 kg/liter water and the amount of lime was 135 ± 130 g/liter. The cooking time for the nixtamal from Santa Maria de Jesus was 74 ± 22 min and for Patzicia was 67 ± 18 min. After cooking, the pH of the lime solution was 12.4 ± 0.2.

To prepare the tortillas, approximately 50 g of the nixtamal corn dough was patted out flat and cooked on both sides on a metal or ceramic plate. Tortilla thickness and size was at the discretion of each individual household. Cooking temperatures typically vary from 170°C on the outer edge to 212°C at the center of the plate *(3)*.

Fumonisin analyses. FB_1 and AP_1 (Table 1) were found in tortillas from both communities. The mean FB_1 contents in tortillas from each of the two communities were not statistically different. However, there was significantly more AP_1 in tortillas collected from Santa Maria de Jesus than from Patzicia (Table 1). The amounts of FB_1 and AP_1 in tortillas from households in Santa Maria de Jesus were, respectively, 0.85 ± 2.0 and 26.1 ± 38.5 μg/g dry weight, and from households in Patzicia were 2.2 ± 3.6 and 5.7 ± 9.4 μg/g dry weight. The fumonisin content ranged from undetectable (<0.4 μg/g dry weight) to 6.5 μg/g dry weight for FB_1, and undetectable (<0.4 μg/g dry weight) to 185 μg/g dry weight for AP_1 in the tortillas from Santa Maria de Jesus. The highest amounts found in tortillas from households in Patzicia were 11.6 μg FB_1/g dry weight and 31.7 μg AP_1/g dry weight.

The frequency distributions for FB_1 and AP_1 in tortillas from Santa Maria de Jesus and Patzicia are shown in Figure 1A and 1B. None of the samples from Santa Maria de Jesus contained ≥10 μg FB_1/g dry weight; two of the samples from Patzicia (6%) had ≥10 μg FB_1/g dry weight. In contrast, Santa Maria de Jesus had a higher percentage of samples with ≥10 μg AP_1/g dry weight (25 samples, or 66%) than Patzicia (10 samples, or 29%). Six of the tortilla samples from Santa Maria de Jesus contained ≥40 μg AP_1/g dry weight (Fig. 1B).

For comparison, the nixtamal from Santa Maria de Jesus contained 13.6 ± 12.5 μg FB_1/g dry weight, with the highest contamination being 77.2 μg/g dry weight. Nixtamal from Patzicia contained 4.4 ± 0.9 μg FB_1/g dry weight, with the highest amount being 5.0 μg FB_1/g dry weight. These differences between the two locations were highly significant ($P < 0.0001$). The frequency distribution (Fig. 1C) illustrates that a high percentage (57%) of the samples from Santa Maria de Jesus contained ≥10 μg FB_1/g dry weight, whereas none from Patzicia had this amount. AP_1 was not detected in the nixtamal from either region.

DISCUSSION

Santa Maria de Jesus and Patzicia are rural Kaqchikel-speaking Mayan communities that differ in prosperity. At the time of this study, in 1995, Santa Maria de Jesus had a chronic water shortage and the city water supply was erratic. Households rationed their water supply, which could impact the local nixtamalization procedure. Since 1995, conditions in Santa Maria de Jesus have improved markedly. Patzicia is an agricultural community in transformation. In the last two decades it has been involved in labor-intensive production of nontraditional cash crops for both the international and local markets, while still producing corn for local consumption. This nontraditional farming has allowed farmers to increase land use and to employ large numbers of farm workers, thus increasing the economic stability of the area. The municipal water supply still has a serious problem but most of the population can afford mechanical wells for household use. Thus, Santa Maria de Jesus and Patzicia were selected as villages with differing socioeconomics but where corn is produced locally and tortillas remain a major food staple.

The current study demonstrates that the fumonisin content of tortillas from both of these rural areas of Guatemala is considerably higher than has been previously reported for tortillas or tortilla chips *(16)*, perhaps because economic conditions have necessitated utilization of lower quality corn. A substantial percentage of the tortillas contained ≥10 μg total fumonisins (the sum of FB_1 plus AP_1 was 66% and 34% in Santa Maria de Jesus and Patzicia, respectively).

FB_1 and AP_1 were present in substantial amounts in tortillas from both regions, and the most heavily contaminated samples contained amounts of FB_1 and AP_1 that are known to be toxic when fed to other animals. The presence of high amounts of fumonisins in tortillas from these communities was confirmed by the finding of FB_1 in nixtamal. It was somewhat surprising, however, that the amounts of FB_1 in nixtamal from Santa Maria de Jesus were significantly higher than for nixtamal from Patzicia, whereas, there was no significant difference in the amounts of FB_1 in tortillas from these two villages (Santa Maria de Jesus did, however, display the highest amounts of AP_1 in tortillas). It was also puzzling that the alkali conditions used in nixtamalization of corn did not hydrolyze all of the FB_1, and that AP_1 was not detected in the nixtamal from either village. These discrepancies may reflect losses during extraction or storage of the samples; however, it is also possible that at least some of the AP_1 found in tortillas was formed from FB_1 after preparation of the masa flour, perhaps during cooking. These questions warrant further study.

Information on corn consumption in the two areas of Guatemala is difficult to determine because of increased poverty in this decade. Nonetheless, the Institute of Nutrition of Central America and Panama has estimated that the average number of tortillas consumed daily in rural indian communities is 10 and 15 for adult women and men, respectively. The average weight of a tortilla is 40 g and thus the average daily consumption is about 400 g and 600 g for women and men, respectively. Based on the findings of this study, the average adult male resident of Santa Maria de Jesus is exposed daily to a mean of 0.51 mg of FB_1 and 15.7 mg of AP_1 and in Patzicia to a mean of 1.3 mg of FB_1 and 3.4 mg of AP_1. Little is known about the toxicity of fumonisins for humans. Thiel et al. *(23)* have estimated that in the regions of the Transkei, South Africa, where fumonisins have been associated with esophageal cancer, a typical 60-kg man consumes an average of 26 mg of FB_1 per day. Compared with the high risk regions of South Africa, the amount of FB_1 consumed by the average adult male in Santa Maria de Jesus and Patzicia is lower. Although, the sum of FB_1 and AP_1 is still quite high (5 to 16 mg/day).

The toxicity of FB_1 has been well established for several animals *(17)*. The toxicity of AP_1 is not as clearly defined as for FB_1 *(7, 10, 25)*. Voss et al. *(25)* concluded that nixtamalization of *F. moniliforme* culture material reduced, but did not eliminate, its toxicity to rats. Furthermore, both corn culture material containing FB_1 and nixtamalized corn containing AP_1 have been reported to in-

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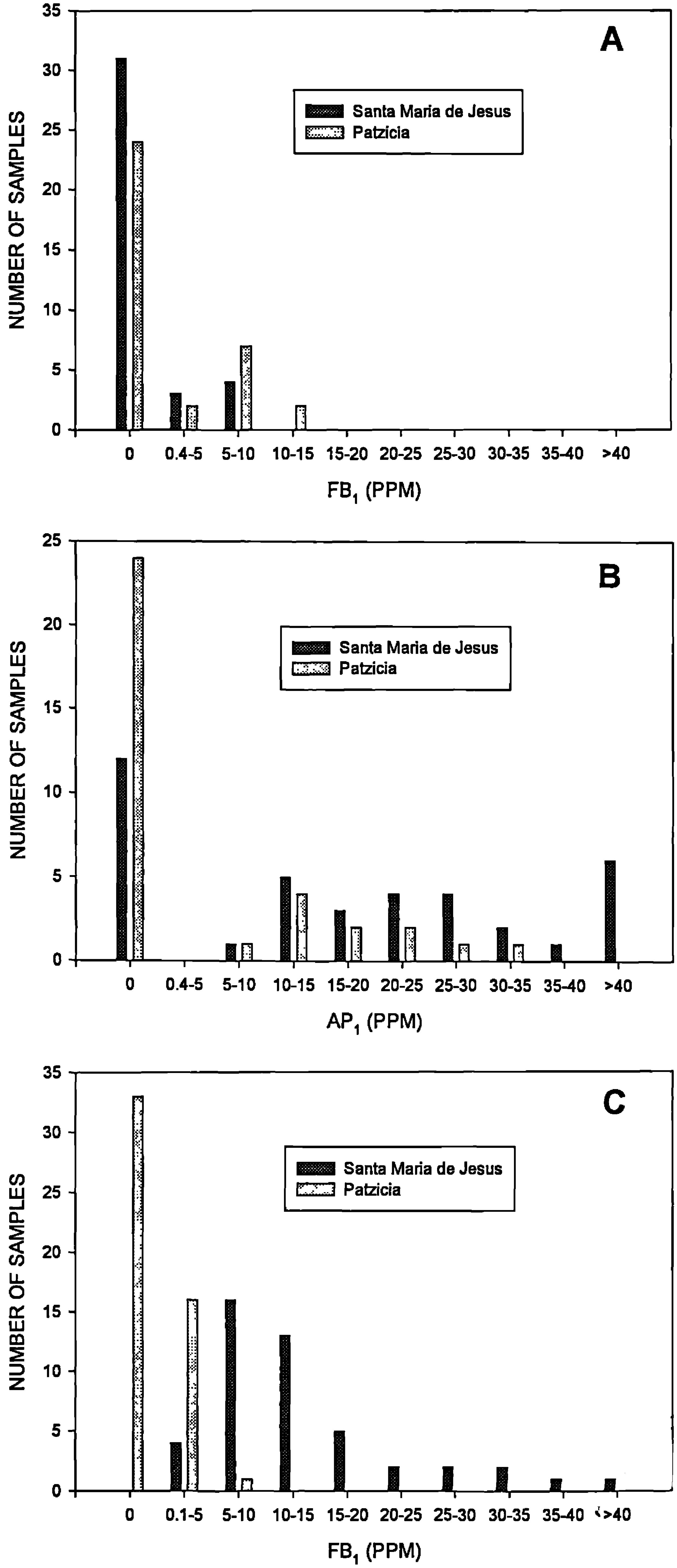
FIGURE 1. *Frequency distribution of (A) fumonisin B_1 (FB_1) and (B) hydrolyzed fumonisin B_1 (AP_1) contamination in tortillas from Santa Maria de Jesus* (n = *38) and Patzicia* (n = *35), and (C) fumonisin B_1 (FB_1) contamination of nixtamal from Santa Maria de Jesus* (n = *46) and Patzicia* (n = *50), Guatemala.*


A
Santa Maria de Jesus
Patzicia
NUMBER OF SAMPLES
0 0.4-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 >40
FB1 (PPM)
B
Santa Maria de Jesus
Patzicia
NUMBER OF SAMPLES
0 0.4-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 >40
AP1 (PPM)
C
Santa Maria de Jesus
Patzicia
NUMBER OF SAMPLES
0 0.1-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 >40
FB1 (PPM)

crease the incidence of liver and kidney lesions in rats exposed to diethylnitrosamine as a cancer initiator *(7)*. The pathophysiology for AP_1 may be complex because it is a less potent inhibitor of the primary cellular target for fumonisins, ceramide synthase *(18)*. However, a recent study has shown that AP_1 can be acylated to *N*-palmitoyl-AP_1, which is more toxic than FB_1 for cells in culture (HT29 cells) *(9)*.

Based on the amounts of FB_1 and AP_1 found in tortillas from Guatemala, fumonisin contamination of corn may pose a risk for human health in this region. Because there are very few studies relating fumonisin consumption and human disease, it is difficult to speculate on the possible adverse health effects that could result from the daily consumption of 5 to 16 mg of fumonisins. It is clearly of concern that at least a portion of the population of rural Guatemala ingests amounts of fumonisins that are considered toxic for other animals. Because fumonisins are possible animal carcinogens and associated with human cancers in Africa and China *(4, 11, 24)*, it is important to determine if there is an association with cancer risk in Guatemala. At the Social Security Hospital of Guatemala the most frequently reported cancer was gastric, accounting for 15.5% of the cancer cases *(21)*. When segregated by sex, gastric cancer is highest in males, accounting for 20.8% of the cases of cancer, and in women it is the third most frequent cancer accounting for 5.8% of the total cancer incidence. The incidence of esophageal cancer is also high in men *(21)*. Additional studies to determine the possible consequences of fumonisin consumption by these two rural populations in Guatemala could improve the understanding of whether or not consumption of fumonisin-contaminated corn and corn products poses any risk to humans.

ACKNOWLEDGMENTS

We thank Philip Stancel, Faustina Bulux, for excellent and expert technical assistance, and Drs. Joy Stallings, K. A. Voss, W. P. Norred, and D. Schroeder for discussions on the data.

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