CEMIT-DGICT-UNA

Deoxynivalenol screening in wheat-derived products in Gran Asunción, Paraguay

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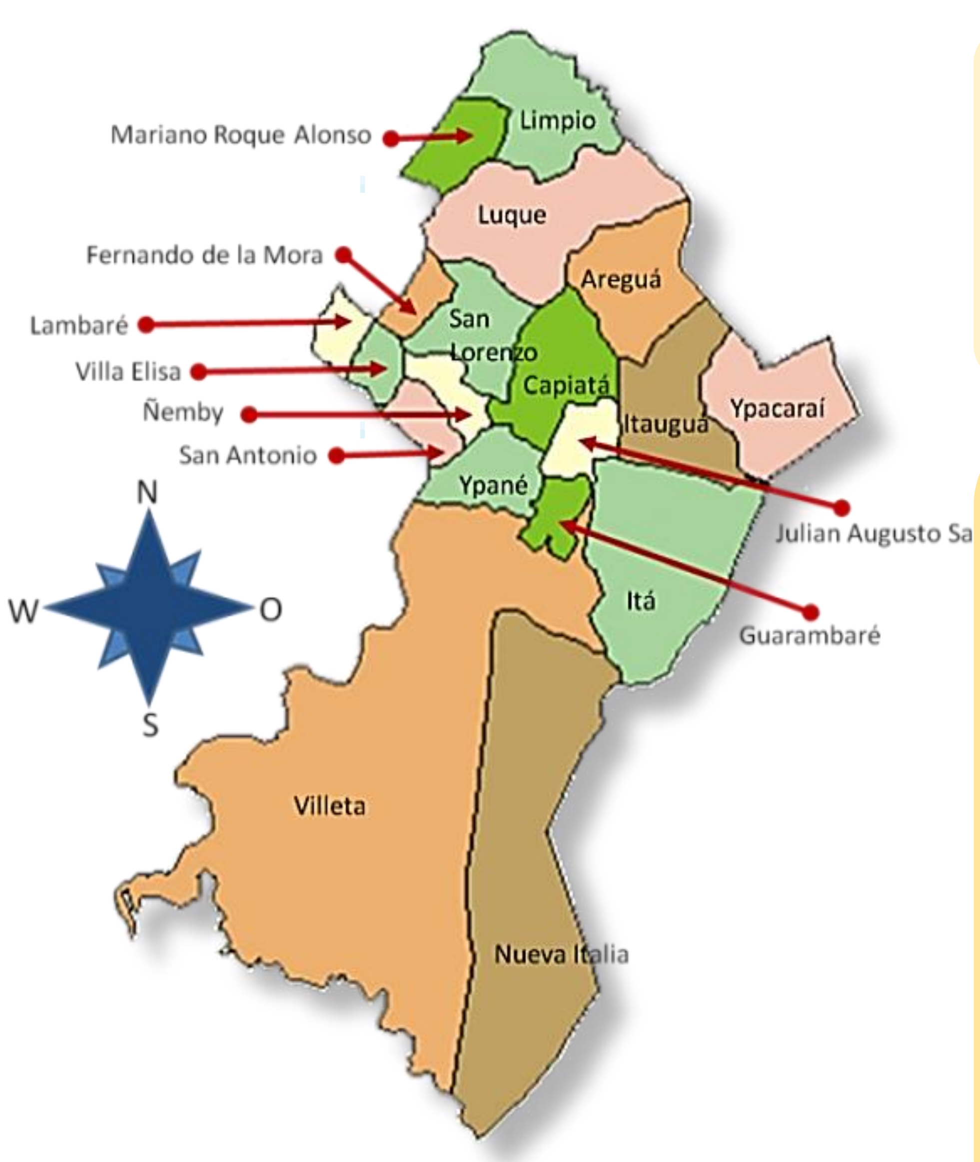
INTRODUCION

Wheat is the main winter agricultural commodity in Paraguay, having reached a key position in the country's productive system, because it contributes millions of dollars to the national economy and stubbles of excellent quality to cover the soil as part of the zero tillage system (Kohli, 2015). During its cultivation, wheat is exposed to the attack of pathogenic microorganisms and one of the most significant phytopathogenic fungi currently associated to crop in Paraguay is *Fusarium sp.* (Quintana de Viedma, 2004), responsible for the disease called Fusarium head blight or white spike. A substantial problem associated with *Fusarium* is mycotoxins, toxic secondary metabolites, responsible for syndromes that occur when ingesting products contaminated by them called mycotoxicosis, both in humans and in other animals (Antonissen et al., 2014; Edite Bezerra da Rocha, Freire, Erlan Feitosa Maia, Izabel Florindo Guedes, & Rondina, 2014). The most important mycotoxins produced by this genus belong to the trichothecene group, being deoxynivalenol (DON) or vomitoxin (Leslie & Summerell, 2006). Gran Asunción concentrates more than 10% of the population of the country; and per-capita consumption of cereals and derivatives is 78 kg per person in the Metropolitan Area, 81 kg in urban areas, and 72 kg in rural areas (DGEEC, 1998). Due to the high consumption of these foods, the population is exposed to the negative effects of mycotoxins, especially within a system where the controls are nonexistent. In Paraguay, during the growing season in the winter, the optimal conditions for the development of the fungus and the production of mycotoxins are achieved; therefore, it is necessary to study the levels of DON present in grains and derived products that are sold in distribution centers in the country. In Paraguay, there is currently no legislation regarding the maximum permissible DON limits in cereals and derivatives, nor any institution to monitor the presence of this mycotoxin in foods. The aim of the present study was to evaluate the presence of DON in wheat products destined for human consumption in Gran Asunción, Paraguay.

MATERIALS Y METHODS



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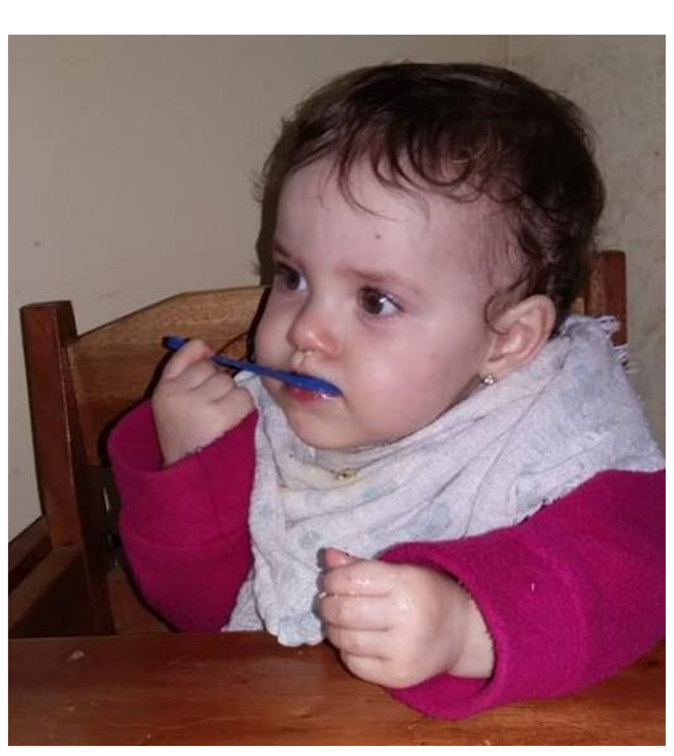
Mariano Roque Alonso

Forty-two samples of food products destined for human consumption were evaluated: four of integral bread, nine of white bread, three of crackers of national production; six of white flour four-zero-type, and 11 of wholemeal flour type from distribution centers of the main cities of the Gran Asunción. Nine samples of imported crackers were also included. Products for export were not considered in this study. 1,000 g of white bread and whole bread were collected; a package was taken from the crackers, and a pack of 1 kg was taken from the flours. All samples were taken in triplicate



Each sample was reduced to 15 g for the detection of DON. For the quantification of DON, 5 g of each product per sample were milled in triplicate. The quantification was done by means of the DON-V Test Strips and the Vertu Lateral Flow Reader (Vicam, Milford, Massachusetts, USA), per the manufacturer's instructions. Detection limit 0.2 ppm; range of lectures 0–10 ppm (Vicam, 2011). The experimental design was completely randomized. Mean concentration of DON was calculated for the different samples processed. The normal Shapiro–Wilk test (Shapiro & Wilk, 1965) was conducted for each type of sample studied. Nonparametric Kruskal– Wallis variance analysis (Kruskal & Wallis, 1952) was used for the comparison of groups with a 95% confidence interval. Mann-Whitney pairwise analysis (Mann & Whitney, 1947) was used to generate p values, which were corrected by sequential Bonferroni test for significance (Brown & Rothery, 1993; Di Rienzo et al., 2011).

RESULTS AND DISCUSSION

The mean results obtained in the measurements are shown on Table 1. Levels of DON were variable. The maximum content of DON was present in imported bran crackers, 11.86 ppm. Regarding the analysis of variance, significant differences were observed in the analyzed samples in relation to the different products and their DON contents. The white bread and domestically manufactured bran crackers (U: 35 and $p < .01$); white bread and imported bran crackers (U: 225 and $p < .05$); white bread and wholemeal flour (U: 225 and $p < .01$); white flour and domestically manufactured bran crackers (U: 27.5 and $p < .01$); wholemeal flour and domestically manufactured bran crackers (U: 14.5 and $p < .05$); imported bran crackers and wholemeal flour (U: 232.5 and $p < .01$); and finally, white flour and wholemeal flour (U: 127.5 and $p < .01$); other comparisons not presented significative differences. In all local products, maximum DON levels remained below those recommended by international regulations (FAO, 2014; FDA, 2010).

TABLE 1 Levels of DON in ppm in wheat derived products for human consumption analyzed in Gran Asunción

	Brown flour	White flour	White bread	Imported bran crackers	Domestically manufactured bran crackers
N	33	18	27	27	9
Min	0.08	0.01	0.02	0	0
Max	1	0.235	0.55	11.86	0.13
Median	0.180	0.103	0.100	0.040	0.020
Mean	0.237	0.114	0.136	1.215	0.038
SD	0.203	0.059	0.099	2.751	0.049

Detection limit 0.2 ppm.

In the results obtained, it can be verified that the DON content was variable and related to the type of product studied. The imported whole-grain crackers had the highest contamination levels with a maximum of 11.86 ppm on average, above international standards that establish a maximum of 1 ppm of DON for products derived from cereals intended for human consumption. In a study carried out in Argentina, 156 samples of different baked products obtained from supermarkets in Luján were studied, and the varying levels of DON were determined by gas chromatography. French bread was the product with the highest levels of DON, ranging between 7 and 271 ng/g. As for bran crackers, DON values ranged between 8 and 85 ng/kg, which were lower than the ones obtained in this study (Pacin, Ciancio Bovier, Cano, Taglieri, & Hernandez Pezzani, 2010). DON is found in wheat grains due to contaminations that occur during its cultivation, being wheat flour one of the main vehicles for the introduction of this mycotoxin to the industry, for the most part as basic raw material to produce various baked goods (Denardi de Souza, Caldas, Primel, & Furlong, 2015). In Brazil, the presence of DON, HT-2 toxin, and T-2 was studied by HPLC-DAD in various types of bread. The presence of DON was detected in 99% of the studied samples, while the percentages of HT- 2 and T-2 were lower—84 and 81%, respectively. In all, 22% of the products studied presented DON levels above those established by Brazilian regulations (Tralamazza, Bemvenuti, Zorzete, de Souza Garcia, & Corrêa, 2016). Also in Brazil, the industrialization process of the wheat grain and its derived baked goods was the object of another study, which concluded that the fraction with highest DON content is bran. In wheat bran and flour, 35% of the samples analyzed showed DON levels above the limits set by regulations instated in Brazil after the study had been performed (Savi et al., 2016). DON is not removed during milling, but is distributed and concentrated in certain fractions; the concentration is lower in fractions destined for human consumption. It has been mentioned that the variation of DON content in the different fractions is due to the difficulty of the fungus to penetrate the grain, therefore the content of the mycotoxin in the external parts is greater as the bran layer behaves like a barrier that prevents the development of the pathogen (Ríos et al., 2009; Wu & Wang, 2015). Similarly to what was observed in this work, DON levels in white flour were significantly lower than those observed in grain and bran. Although it is the first time that this type of study is carried out in Paraguay in products destined for human consumption, in an earlier work carried out on wheat grain, HPLC levels of DON were detected at levels ranging from 0.247 to 10.13 ppm (Quintana de Viedma, 2004). At present, Paraguay lacks any regulations regarding acceptable levels of DON in flour and baked goods, but the United States Food and Drug Administration establishes DON levels of 1 ppm for products derived from wheat intended for human consumption and 5 ppm for products destined for animal consumption. The levels detected are below said limit in all local products analyzed. In this study, the presence of DON in samples of wholemeal bread, white bread, national and imported whole meal cookies, wholemeal flour and white flour was confirmed. It is important to consider the need for a national legislation that establishes acceptable limits of DON in products intended for human consumption, and the implementation of management systems at the field level to prevent contamination with *F. graminearum* and DON.

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