Vol.47, n. 6 : pp. 911-919, November 2004
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Fumonisins B_1 and B_2 in Corn-Based Products Commercialized in the State of Santa Catarina - Southern Brazil

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ABSTRACT

Corn flour, "canjica" (corn grits), corn flakes and popcorn for human consumption, commercialized in Santa Catarina (n=82), were analyzed in order to detect the presence of fumonisins B_1 (FB_1) and B_2 (FB_2). From the samples, 92.68% showed detectable levels of Fumonisins (FBs). Corn flour showed the highest level of contamination (91.5%) with average levels of 3.811 and 5.737 µg/g for the home-processed and industrialized products, respectively. The next most contaminated product was popcorn with a average of 2.872 µg/g and an occurrence in 91.6% of the samples. All samples of corn flakes were contaminated with an average of 1.307 µg/g. The product with the lowest levels of FBs was "canjica" with a average contamination of 0.732 µg/g. These results indicated the need of monitoring corn-based products in this state.

Key words: Corn, fumonisins, Brazilian corn products

INTRODUCTION

Fumonisins**,** mycotoxins produced by *Fusarium* sp (*F. verticillioides* and *F.proliferatum*) are found chiefly in corn and corn-based products. In this group, the mycotoxin that most commonly contaminates corn naturally is the fumonisin B_1 (FB_1), which may be found in either grains used for human and animal consumption.

Since its discovery (Bezuidenhout et al.,1988) fumonisin has been associated with diseases in animals, such as LEME in equine (Kellerman et al.,1990), pulmonary edema, hydrothorax and cardiovascular disturbances in pigs (Harrison et al., 1990, Gumprecht et al., 2001), including nephrotoxic, hepatotoxic and imunosupressing effects, in various animal species (Marasas, 1996). Although their effect on humans is still not clearly established, statistically it is associated with esophageal cancer (EC) in some regions such as Transkei/ South Africa (Sydenham et al., 1990; Rheeder et al., 1992), Linxian/China (Chu et al., 1994). FBs are considered possible carcinogenic for human, Group 2B (IARC, 2002). Francheschi et al. (1990) linked the rise in the development of EC in north-eastern Italy to increased corn consumption, in which *polenta* is the main basic food (Doko and Visconti, 1994) with a possible relation to a human carcinogenic In Brazil, it has been related to outbreaks of toxicosis in animals

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(Meirelles et al., 1994; Sydenham *et al*, 1992). There are records of the occurrence of fumonisins in corn and corn-based products, particularly in the Southern and Southwestern regions of the country, with levels that vary from 0.02 to 49.31μg/g for FB_1 and from 0.05 to 29.16 μg/g for FB_2 (Camargos et al., 2000; Hirooka et al., 1996; Ono et al., 2000, 2001, 2002; Orsi et al., 2000).

In Santa Catarina, FBs studies reported up to 97.3 % of the corn sold is contaminated. Hermanns et al. (2000) reported levels that ranged from 1.12 to 31.88 μg/g in corn from the western region. A study involving three regions of the state (West, North and South) by van der Westhuizen et al. (2003) found levels ranging from 0.02 to 18.74 μg/g.

There is a scarcity of publications on fumonisins in corn-based products, destined to human consumption in Brazil. Machinski et al. (2000) analyzed 81 samples of corn-based products, collected in Campinas (SP), where fumonisins were found in 54 % of the samples with levels between 0.02 and 4.93 μg/g. In a study carried out in Argentina, Hennigen et al. (2000) analyzed 35 samples of corn flour and corn grits, destined to human consumption and found fumonisins in 91.4 % of the products. The levels varied from 0.038 to 4.987 μg/g and from 0.015 to 1.818 μg/g for FB_1 and FB_2, respectively.

These facts show that it is imperative to evaluate the risks to which the population is exposed considering, among other aspects, that the Southern Region of Brazil shows the greatest incidence of EC in the country (INCA, 1999), and that fumonisins could be one of the factors related to this problem (Scaff and Scussel, 1999; Bolger et al., 2002). In Santa Catarina, this is the first evaluation of the levels of fumonisins in corn-based products destined to human consumption, particularly corn flour.

MATERIAL AND METHOD

Samples: Home-produced and industrialized corn flour (47), pre-cooked corn flakes (11), canjica (corn grits) (12), popcorn (12), (total: 82 samples - 1 kg), commercialized in different regions of SC (North, Itajai Valley, Florianópolis, Mountain Region, West and South), but processed in the Central-West, Southeast and South of Brazil were collected from January to June of 2001 according to official methodology of the Ministry of Agriculture (BRASIL, 1976). The products were collected from supermarkets, mills and cooperatives, in different regions of the state.

Samples preparation

Each sample was ground in mill (250 μm mesh), homogenized and reduced through the technique of quartering up to 250 g (sub-sample) and from this, 50 g portions were removed for analysis.

Analysis of fumonisins

AOAC official method n° 995.15 (AOAC, 2000), based on Shephard et al. (1990), validated in a collaborative study by Sydenham et al. (1996) was used methanol:water (3:1) was utilized as extraction solvent and SPE cartridges - SAX were used for cleaning the samples. Derivatization of FBs was carried out with OPA and detection by HPLC, with a fluorescence detector (335 nm of excitation and 440 nm of emission). An isocratic mobile phase was used (methanol:sodium di-hydrogen phosphate buffer - 0.1M (77:23). Flow rate: 1 ml/min. For calibration, fumonisin standard solutions were utilized at concentrations of (a) 0.0125; 0.025 and 0.05 μl/ml for FB_1 and (b) 0.005; 0.01 and 0.02 μg/ml for FB_2, (ACN:H_20/ v:v). The limit detection of method (LDC) was 0.04 μg /g to FB_1 and 0.05 μg /g for FB_2. The method recovery was of 88% and 69% for FB_1 and FB_2 respectively. The data were analyzed through simples and partial correlation analysis using statistical software (SAS, version 6.11)

RESULTS AND DISCUSSION

The data referring to corn-based products (corn flour, *canjica*, corn flakes and popcorn), commercialized in SC, but processed in different states (Goiás, São Paulo, Paraná and Santa Catarina) is shown in Tables 1and 2. Fumonisins were found in 92.6 % of the samples (76/82) with levels that ranged from 0.060 to 21.823 μg/g. All the contaminated samples contained FB_1 but FB_2 was only detected in 42.6 % of the products.

According to the recommendation of the Food and Drug Administration (FDA, 2001) and Trucksess (2001), the maximum residues level (MRL) for FB_1+FB_2+FB_3 permitted in foods for human consumption is 4 μg/g but 29.2 % of the contaminated samples in our study showed higher

levels of fumonisins, referring only to the summing of FB_1+FB_2.

(a) Corn Flour

The product with the highest contamination level was corn flour (*fubá*), stressing the fact that 22 samples (46.8%) of these samples came from (a.1) *home processed* for their own or regional consumption, produced in conditions of hand labor, minimally processed, from the whole grain, maintaining the germ. The other 25 samples (53.2%) were (a.2) flours *processed industrially*, without the germ (Table 1).

(a.1) Corn flour home produced: Only one sample did not show contamination by fumonisins and the levels were between 0.086 and 19.222 μg/g with an average of 3.811 μg/g. The majority of these products (18 samples) was collected in the South and West of SC, agricultural regions of the state, where were normally produced and consumed. They showed marked cultural characteristics of the Italian colonization, since they were the usual consumers of *polenta*. In the South, higher levels were found (up to 19.222 μg/g) for FB_1 + FB_2, as well as a higher frequency when compared to the West (up to 10.699 μg/g) (Table 1).

(a.2) Industrialized corn flour: Of the samples analyzed 92 % showed contamination and the levels ranged from 0.145 to 21.823 μg/g with an average value of 5.737 μg/g. The highest contamination levels of the product were also found in the South of the state where all the samples analyzed were contaminated. The samples with the highest levels of contamination were found in products from SC, although expressive levels have been detected in products from the states of Goiás and São Paulo.

Since *home-processed corn flour* is prepared with the whole corn and, higher contamination be expected in this product, but no significant difference was observed between the results of these two types of corn flour. Considering that in rural areas the intake of corn-based products may vary from 11 to 39g per person/day (Machinski et al., 2000), these levels represent a health risk for the population. Thus, in these regions the possible intake of fumonisins to which this population would be exposed would be much higher than the level proposed by Gelderblom et al. (1995), in which the Tolerable Daily Intake is 800ng/kg bw/day.

The results shown here reinforced previously cited data, although in this case they refered to corn-based products, destined to human consumption and not only corn, where levels ranged from 1.12 to 31.88 μg/g (Hermanns et al., 2000) and between 0.02 and 18.74 μg/g (van der Westhuizen et al., 2003) for fumonisins. In Brazil some publications, mainly in the states of the South and Southeast, pointed to the presence of fumonisins in corn (table 3) with a very high frequency of samples with positive and mean levels generally over 4 μg/g. Machinski et al. (2000) found contamination by fumonisins in all the samples of corn flour analyzed (9/9) with levels ranging from 0.56 to 4.93 μg/g, and an average of 2.32 μg/g. The average levels found in our analyses were 5.165 μg/g, higher than the above, which demonstrated the urgency of monitoring the product in our region.

(b) Other products: Popcorn, corn flakes, *canjica*

The average contamination levels by fumonisins B_1 + B_2 in *canjica*, corn flakes and popcorn were relatively lower than those detected in corn flour, but some samples showed levels that caused concern (Table 2).

(b.1) Popcorn: The second most contaminated product was popcorn showing FB_1 ranging from 0.102 to 7.346 μg/g (frequency of 11/12) and of 0.317 to 2.427 μg/g for FB_2 (6/12). The average contamination levels were 2.872 μg/g. Machinski et al. (2000) found contamination levels in popcorn in the state of SP from 0.300 to 1.72 μg/g, lower than those found in our study. According to Bolger et al. (2001) in Argentina fumonisins in popcorn were detected, ranging from 1.084 to 14.241 μg/g, in 42 samples collected between May and June of 1999.

(b.2) Corn flakes: Fumonisins were found in all of the samples, with an average contamination of 1.307 μg/g. Since this product, in addition to being ground, was submitted to thermal processing (cooking), reduction of FBs was expected, either by removal or by degradation. The grinding process, dry or moist, may lead to removal of the mycotoxin, more significant, in the latter case, (Bolger et al., 2001). The effect of the thermal processing is still being widely discussed, but no definite conclusion has been reached. There are studies that indicate reduction (Jackson et al., 1996) while others show thermal stability of

fumonisins (Alberts et al., 1990; Dupuy, 1993). According to Bolger et al. (2001), temperature > 150°C is necessary for a significant loss to occur. Nevertheless, there are cases recorded in which products thermally processed generally show lower levels of FBs than those not processed or minimally processed (Stak and Eppley, 1992).

(b.3) Canjica: These products showed lowest contamination levels, with FB_1 ranging from 0.297 to 2.237 μg/g and occurrence in 83 % of the samples and FB_2 detected in only one sample at a level of 0.098 μg/g. The average contamination of this product was 0.732 μg/g.

In Santa Catarina the results indicate the need for control and monitoring of corn and corn-based products, destined to human consumption, in view of the regional characteristics of communities of descendents from Italian immigrants, habitual consumers of polenta, a basic food, prepared from corn flour (*fubá*), minimally processed.

Table 1 - Fumonisins in corn flour commercialized in SC State, Southern region of Brazil

Corn flour [a]	**Processed**		**Commercialized**	**Fumonisins (μg.g^{-1})**		
	Region	**State**	**Region**	**FB_1**	**FB_2**	**FB_1+FB_2**
Industrialized *	Central-West	GO	Florianópolis	1.670	ND	1.670
				2.116	0.464	2.58
				9.062	2.591	11.653
				11.433	3.404	14.837
	Southeast	SP	Northern	0.430	ND	0.430
				0.561	ND	0.561
			Itajaí Valley	0.834	0.067	0.901
				1.446	ND	1.446
				4.365	ND	4.365
				9.944	2.840	12.784
	Southern	PR	Northern	ND	ND	ND
			Florianópolis	0.145	ND	0.145
				0.336	ND	0.336
				0.717	ND	0.717
		SC	West	ND	ND	ND
				3.008	0.365	3.373
				3.825	1.010	4.835
			Southern	3.958	1.107	5.065
			West	4.373	1.157	5.530
				5.659	1.326	6.985
				7.631	2.024	9.655
			Southern	9.435	3.047	12.482
				11.68	3.845	15.525
				16.357	5.466	21.823
				16,455	4,79	21,245
			Range	0.145 - 16.455	0.067 - 5.466	0.145 - 21.823
Total: 25			Average	5.453	1.196	5.737
Home processed ♦	Southern	SC	Itajaí Valley	0.255	ND	0.255
				0.506	ND	0.506
				2.559	0.676	3.235
			West	ND	ND	ND
				0.086	ND	0.086
				0.411	ND	0.411
				0.709	ND	0.709
				1.082	ND	1.082
				2.540	ND	2.540
				4.767	0.475	5.242
				5.379	1.398	6.777
				8.526	2.173	10.699

Cont. Table 1

Cont. Table 1

		Mountain	3.899	0.060	3.959
		Southern	0.750	ND	0.750
			0.265	ND	0.265
			0.293	ND	0.293
			0.531	ND	0.531
			1.304	0.237	1.541
			4.185	1.050	5.235
			6.013	1.559	7.572
			10.074	2.871	12.945
			15.038	4.184	19.222
		Range	0.086 - 15.038	0.060 - 4.184	0.086 - 19.222
Total: 22		Average	3.144	0.667	3.811

[a*] different types
[*] degerminated
♦ with germe
ND not detected
GO, SP, PR, SC: Goiás, São Paulo, Paraná and Santa Catarina

Table 2 - Fumonisins in corn products, commercialized in SC State, southern region of Brazil

Corn product [a]	Processed		Commercialized	Fumonisins (µg.g^{-1})		
	Region	State	Region	FB_1	FB_2	FB_1+FB_2
Canjica	Southeast	SP	Itajaí Valley	ND	ND	ND
				0.533	ND	0.533
				2.237	ND	2.237
	Southern	PR	West	0.706	ND	0.706
		SC	Itajaí Valley	ND	ND	ND
				0.531	ND	0.531
			Mountain	0.730	ND	0.730
			West	0.297	ND	0.297
				0.536	ND	0.536
				0.818	ND	0.818
				0.943	ND	0.943
				1.358	0.098	1.456
			Range	0.297 - 2.237	0.098*	0.297 - 2.237
		Total: 12	Average	0.724	0.008	0.732
corn flakes	Central-West	GO	Florianópolis	1.419	ND	1.419
	Southeast	SP	Itajaí Valley	0.383	0.113	0.466
				0.473	0.084	0.557
				1.094	ND	1.094
			Florianópolis	0.797	ND	0.797
				0.807	ND	0.807
				4.528	1.328	5.856
	Southern	SC	North	0.980	ND	0.980
				0.157	ND	0.157
				1.125	ND	1,125
			West	1.115	ND	1.115
			Range	0.157 - 4.528	0.084 - 1.328	0.157 - 5.856
		Total: 11	Average	1.170	0.139	1.307
Popcorn	Southeast	SP	Florianópolis	ND	ND	ND
			Itajaí Valley	0.139	ND	0.139
			South	2.699	0.317	3.016
	Southern	SC	Itajaí Valley	6.386	1.652	8.038

Cont. Table 2

Cont. Table 2

	West	0.237	ND	0.237
		0.600	ND	0.600
		2.234	0.593	2.827
		2.739	0.617	3.356
		7.346	2.427	9.773
	South	0.102	ND	0.102
		2.286	ND	2.286
		3.268	0.824	4.092
	Range	0.102 - 7.346	0.317 - 2.427	0.102 - 9.773
Total: 12	Average	2.336	0.536	2.872

[a] different type
* only contaminated sample
ND not detected
GO, SP, PR, SC: Goiás, São Paulo, Paraná and Santa Catarina

Table 3 - Occurrence of fumonisins in corn of Central-West, Southeast and Southern region of Brazil, cited in literature

Region	State	Range (µg/g)		Mean (µg/g)	FB_{1+} FB_2 (µg/g)	Positive samples	Source
		FB_1	FB_2				
Central-West	Mato Grosso Sul	4.90 - 18.52	3.62 - 19.13	10.59 [b]	NS	100 %	Hirooka et al., 1996
Southeast	São Paulo	0.09 -17.69	0.05 -5.24	NS	NS	93.5%	Almeida et al., 2002
		0.87 - 49.31	1.96 - 29.16	8.05 [b]	NS	97.4 %	Orsi et al., 2000
		1.63 -25.69	0.38 - 8.60	5.61 [b]	NS	100 %	Camargos et al., 2000
Southern	Paraná	0.60 - 12.55	1.20 - 10.24	4.79 [b]	NS	97.4 %	Hirooka et al., 1996
			NS	2.39 [b]	0.07 - 13.46	100 %	Ono et al., 2000
		NS	NS	9.85 [x] 5.08 [y] 1.14 [z]	0.096 - 22.60	98 %	Ono et al., 2001
		NS	NS	9.9 ± 6.0	0.74 - 22.6	100 %	Ono et al., 2002
	Santa Catarina	1.12 - 31.88	NS	NS	NS	100%	Hermanns et al., 2000
		NS	NS	2.66 ∀ 2.73	0.02 - 18.74	100 %	Westhuizen et al., 2003
	Rio Grande do Sul	0.086 - 78.92	NS	8.86 [b]	NS	35.2 %	Mallmann et al., 2001

NS not specified
[b] Fumonisin FB_1
[x] Northern
[y] Central-Western
[z] Central-Southern

Knowing that southern Brazil shows a relatively high incidence of esophageal cancer (EC) and that the presence of fumonisins is related to this pathology in some regions of the world, associated with smoking, consumption of alcoholic beverages and "*chimarrão*" (an infusion of green leaf consumed hot) (Perin et al., 1990; Scaff and Scussel, 1999) these mycotoxins may be contributing in these statistics, since cancer is now seen as a multifactorial disease.

These data, resulting from regional and partial findings and analyzed along with other results obtained point to a need for frequent studies of a broader scope, which show the overall situation in all Brazil and take into consideration climatic and seasonal differences.

RESUMO

Farinha de milho, canjica, flocos de milho, milho de pipoca, destinados ao consumo humano e comercializados em Santa Catarina (n=82), foram analisados a fim de determinar a ocorrência de fumonisinas B_1 (FB_1) e B_2 (FB_2). Das amostras, 92,68 % apresentaram níveis detectáveis de FBs. A farinha de milho apresentou os maiores níveis de contaminação (91,5%) com níveis médios 3,811 e 5,737 μg/g para as de preparo artesanal e industrializadas, respectivamente. O segundo produto mais contaminado foi o milho de pipoca com uma média de contaminação de 2,872 μg/g e ocorrência em 91,6% das amostras. Todas as amostras de flocos de milho apresentaram contaminação com uma média de 1,307 μg/g. O produto com menores níveis de FBs foi a canjica com contaminação média de 0,732 μg/g. Estes resultados indicam a necessidade de monitoramento dos produtos derivados de milho em nosso Estado, ressaltando-se que os níveis mais expressivos foram encontrados em produtos comercializados no Sul e Oeste de SC, regiões agrícolas, marcadamente colonizadas por descendentes de italianos, consumidores habituais de produtos derivados de milho, particularmente a polenta, preparada a partir de farinha de milho.

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Received: June 25, 2003;
Revised: November 18, 2003;
Accepted: July 20, 2004.