Effect of commercial processing on fumonisin concentrations of maize-based foods

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Abstract

Fumonisin-contaminated maize was used to study the effect of three cooking and food processing methods and residual contamination of the food product. Frying, autoclaving and extrusion were examined with naturally-contaminated maize meal, maize flour and sweet maize kernels, all at two fumonisin concentrations. High Pressure Liquid Chromatography determination of fumonisins B1 and B2 and hydrolized fumonisin B1 (AP1) were performed in unprocessed materials and at the end of the experimental runs. Reductions of fumonisins concentration in processed products were obtained for fried polenta and from one of the two runs of extruded maize batter. These reductions were consistent with previous studies of the thermal degradation of fumonisins. Autoclaving sweet maize kernels apparently resulted in reductions of fumonisin concentrations that were highly temperature dependent, but this needs further study. The unexpectedly large reduction in fumonisin concentrations in the extrusion processing of batter with high initial concentrations also needs further investigation. There was no evidence that AP1 was formed under any of the conditions tested.

Introduction

Fumonisins are mycotoxins produced by *Fusarium verticilloides* (formerly *F. moniliforme*), *F. proliferatum* and related species (1,2). *F. verticilloides* colonizes maize worldwide and causes the most important ear disease of maize, including in South America, fusarium kernel rot (3,4,5,6). In nature, fumonisins B1 and B2 are the dominant compounds accumulated in affected maize worldwide including in South America (7,8,9,10,11,12,13,14,15,16).

Acute exposures to fumonisin cause equine leukoencephalomalacia (17) and porcine pulmonary edema in swine and there are toxic effects at low exposures that affect animal production (2,18,19). Fumonisin B1 and culture material of *F. verticilloides* cause liver tumours in rats and mice (9). Ingestion of maize affected by *F. verticilloides* containing fumonisin is associated with esophageal cancer in the Transkei, South Africa and in northeastern Italy (2,9). Uruguay and southern Brazil have elevated rates of esophageal cancer in rural popluations. This has been associated with drinking "mate", a local herbal infusion sipped very hot through a metal straw (20,21). However, the possible association between fumonisin exposure and esophageal cancer in these populations, for whom maize is a part of the staple diet, has not been studied.

A survey of fumonisin levels in Uruguay's most important maize and maize-based foods demonstrated widespread occurrence in products sampled from local markets (22). Sweet maize and milled maize products such as maize flour, meal or polenta were the foods which had the highest fumonisin concentrations. Grain maize products containing fumonisins are the main nutrient source in Uruguay in much of the population. In addition to fumonisins, deoxynivalenol and zearalenone occur in high prevelance in maize and other grains in Uruguay (23,24,25).

Thermally-processed maize products generally contain lower concentrations of fumonisins than unprocessed products (22,26,27). Several studies have focused on the effect of thermal processing on fumonisins (28,29,30,31, 32,33,34). These suggest that when foods are heated at temperatures encountered in boiling or retorting foods (100-125°C), little change in fumonisin content can be expected. But foods that reach temperatures of >150° during processing (baking or frying) may have some losses of fumonisins.

Another treatment of maize, treating with base to prepare tortilla flours, destroys fumonisin B1 but converts it to the amino pentol product AP1. Some or possibly all of the toxicities of FB1 are retained by the breakdown product AP1 (35,36). Thermal degradation of fumonisin produces breakdown products including, possibly, AP1 (33).

The objective of the present study was to investigate the effects of three commonly used maize processing methods on the content of fumonisins and AP1 in the products: frying of maize meal, extrusion of maize flour, and autoclaving of sweet maize kernels.

Materials and methods

Sampling and sample preparation:

The source of naturally contaminated samples for maize meal and flour was pooled material of the 5 most popular national brand products (San Salvador, Manzanares, Puritas, Cefa, Adria). These were purchased and sampled at random from local commercial retail markets, five packages of each type for a total of 3 kg. They were homogenized in a Romer mill prior to analytical subsampling.

Naturally contaminated maize samples were collected from four different fields in different geographical areas of Uruguay (Colonia, Mercedes, Canelones and San Jose), 3 kg from each, mixed and homogenized in a Romer mill into one batch prior to subsampling for processing.

Determination of fumonisin contamination:

The natural contamination of sweet maize, maize meal and flour was determined by the liquid chromatography method described below. Depending on the values obtained, each product was grouped into low and higher concentration lots (levels 1 and 2),

blended and homogenized, and, five determinations were done of the final pool for fumonisins concentrations. Results were averaged for each level and these taken as the unprocessed contamination levels. These two batches for each food product were used for all the processing trials.

All values are expressed as a dry weight basis.

Design of experimental runs:

A minimum of three replicates for both levels were analyzed for each of the three technological processes studied: frying, autoclaving and extrusion. Three experimental runs were carried out for each of the processes in the LATU pilot processing facilites. Except for the sweet maize kernels in brine, the pH was neutral (pH=7). Three fumonisin-free (<8ng/g for FB1 and FB2 and <60 ng/g AP1) controls were included in each process run. Pre-process baseline blanks were analyzed for fumonisin content. Spiked samples prepared from fumonisin-free sweet maize kernels, meal and flour were assayed for method recovery with recoveries ranging from 72% to 100%. Analyses of fumonisin B1, fumonisin B 2 and AP 1 were performed in all raw materials and at the end of the experimental runs.

Liquid chromatography determination of fumonisins:

FB1, FB2 and AP1 were analyzed according to the method of Scott et al. (37). The 10g ground samples were blended and extracted with methanol-water. Extracts were cleaned on a strong anion exchange (SAX) solid phase extraction column (SPE) and then for AP1 on a C18 SPE column. Analyses were done by reverse phase High Performance Liquid Cromatography and OPA derivatization with fluorescence detection in a Hewlett Packard 1050 isocratic system with 20 µl sample loop, 100x4.6 mm C18 ODS Hypersil column with 5 µm particle size and precolumn; Hewlett Packard fluorescence detector Model 1046A with excitation wavelength of 232 nm and emission wavelength of 425 nm; and Hewlett Packard Model 1050 integrator. The mobile phase consisted of Solution B-methanol HPLC grade (70:30) where solution B was solution A-water HPLC grade (9:1) adjusted to apparent pH 3.3; and solution A was methanol HPLC grade-0.1M sodium dihydrogen phosphate (77:23).

Confirmation of AP1:

Confirmation of AP1 was performed at the Center for Food Safety and Applied Nutrition, US FDA by LC/Mass Spectrometry to a detection limit of 100 pg. The samples were analyzed on a Finnigan Model TSQ-700 triple quadrupole using electrospray ionization.

Fumonisin Standards:

FB1 and FB2 standards were purchased from Sigma Chem. Co. (St. Louis, Mo.) and AP1 was a gift from M.Stack.

Statistics:

For each process and each level, the ratio of FB1 and FB2 concentration of the treated samples to initial content of the untreated samples (C/C0) was calculated to evaluate reduction. Statistical evaluation of the data was performed by ANOVA analysis of variance and Tukey's multiple comparison test to determine significant differences in treated and untreated samples.

Commercial maize processing methods:

Frying - Maize meal or "polenta" containing final mean values $\pm$ standard deviation of

2388±65.1 ng/g FB1, 227±36.9 ng/g FB2 and <60 ng/g AP1 for level 1 and 8955±2114.6 ng/g FB1, 2345±301.1 ng/g FB2 and 72 ng/g AP1 for level 2, was processed in the traditional Italian style by frying polenta squares with a moisture content of 82 % final in maize oil at 160°C for 3 minutes in three separate runs.

The polenta squares were hand made by mixing 350g of maize meal in 400mL cold water and adding to 1600mL hot water and heating to 100°C for 15 min. The wet weight of an average square was 42g ± 3.6 (n=10), corresponding to an average dry weight of 33g ± 2.6 (n=10).

After the squares were removed from the oil, they were placed on paper towels until cool (20 min). They were mascerated in a Waring blendor to an oily paste.

Autoclaving - Naturally contaminated sweet maize kernels containing 549±65.2 ng/g FB1, 223±14.7 ng/g FB2 and <60 ng/g AP1 for level 1 and 2090±15.5 ng/g FB1, 258±13.5 ng/g FB2 and 64 ng/g AP 1 for level 2, with brine (5g NaCl/L) added were autoclaved by commercial sterilization of cans for 28 min at 121°C, 15 lbs pressure, in three separate runs.

An amount (30g) of sweet maize kernels were placed in 250mL mason jars with 100 mL brine for autoclaving. The unautoclaved kernels were held at room temperature for about 3 h and they gained some moisture during this period (ca. 15%). The autoclaved kernels absorbed all the brine. A correction was made for the salt when adjusting for dry weight. The pH of the maize-brine mixture was 5.4 and was not affected by autoclaving.

Extrusion - Extrusion of maize flour containing final average values of 523±23.7 ng/g FB1, 66±0.8 ng/g FB2 and <60 ng/g AP1 for level 1 and 1119±6.1 ng/g FB1, 199±10.8 ng/g FB2 and 64 ng/g AP1 for level 2, with a moisture content of 14%, was performed in three separate process runs. A single screw extruder (Brabender Laboratory Extruder 20DN, Germany) was used set to the following parameters:

Screw: 2:1 - 5:1 and 3mm round tube die
Screw speed: 200 turns min-1
Feeding screw speed: 30 turns min-1
Screw compression: 4:1
Pressure: 58, 55, 60 psi (for runs 1, 2, 3)
Torque: 58, 60, 40 Nm (for runs 1, 2, 3)
Temperature: zone 1: 150°C
zone 2: 180°C
zone 3: 180°C
The samples were ground in a Waring blendor to a dry powder.

Results and Discussion

The fumonisin values obtained for the three replicate samples and for the three runs were averaged for each process and compared to the average fumonisin values in the raw materials for both concentration levels (Figure 1). Fumonisin reductions in processed products occurred during frying and extrusion, particularly in extruded batter with high initial amount of fumonisins (Level 2). Results were less consistent for autoclaving but also suggested some reduction.

Frying resulted in statistically significant reductions of FB1 and FB2 average concentrations by approximately 80%, with the percent FB1 or FB2 remaining (C/C0 x

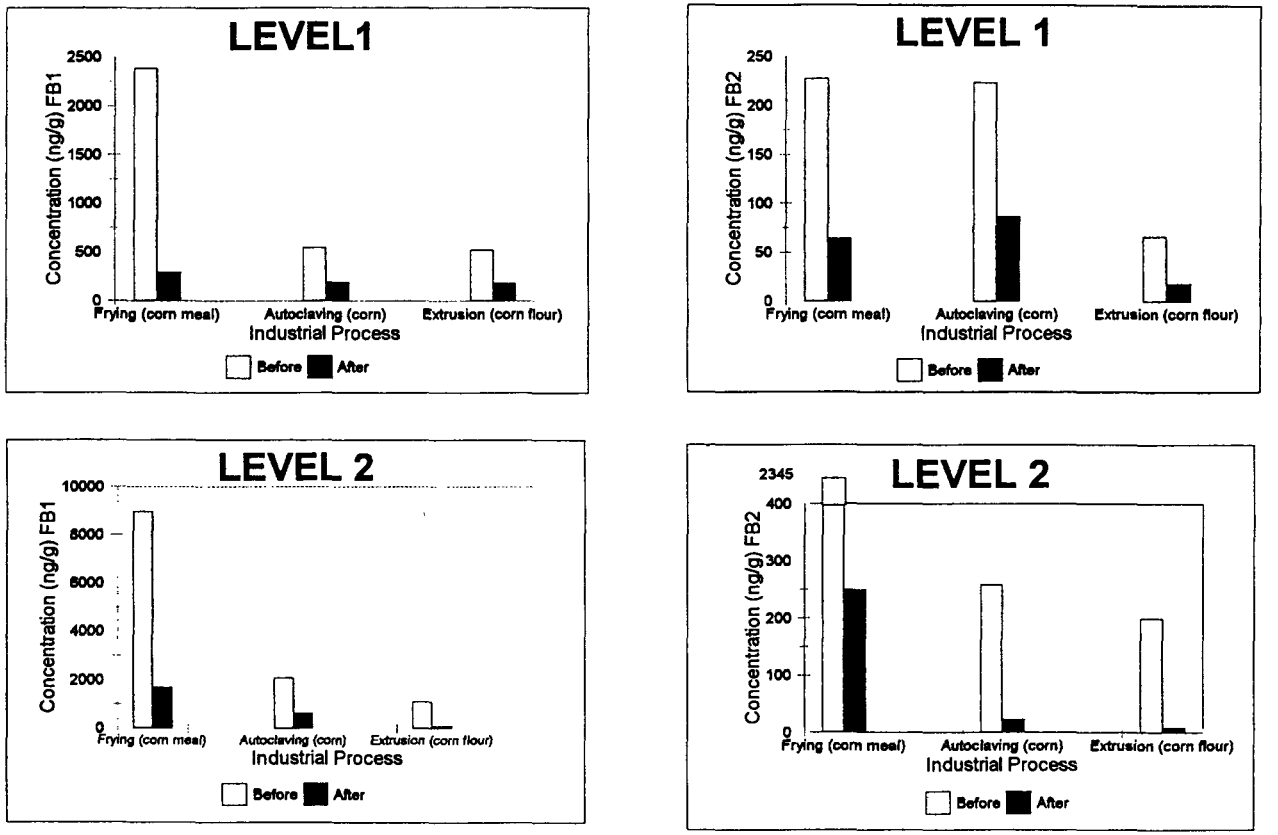

Figure 1. EFFECT OF PROCESSING METHODOLOGIES ON FUMONISIN CONTENT*

*Each point represents the average of 3 replicates (minimum) and 3 runs

100) ranging from 8.6% to 32%, at both the high and low initial concentration of fumonisins in the maize meal (Table1). The extent of degradation of FB1 and FB 2 were similar to that reported by Jackson et al (33,34). No AP1 was detected in these samples. Analyses of replicate samples gave moderate to high deviation. This is thought to be due to the difficulties of obtaining representative samples from the oily paste that was obtained for the fried product. Based on studies of fumonisin degradation in fungal-maize cultures, the extent of thermal degradation by frying was approximately twice that with dry heat (29,30).

Autoclaving sweet maize kernels resulted in statistically significant reductions in FB1 and FB2 concentrations of approximately 70%, with %C/C0 ratios of 20.8 to 35.1 in the high initial concentrations of fumonisins (Table 2). The extent of degradation of FB1 and FB2 was similar. Although not significant, the trend was the same in the low initial fumonisins concentration batches. No AP1 was detected in these samples. Analyses of replicate samples gave moderate deviation. Differences between runs were explained by differences in the autoclave process. Unexpectedly, the autoclave runs were not identical although the sterilization criteria (28 min, 121°C, 15 lbs pressure) were met.

Table 1 RATIO OF OBTAINED CONCENTRATION /INITIAL CONCENTRATION (C/Co)

CORN MEAL - FRYING - LEVEL 1

		% C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB1	NQ	7.7	13.0	12.5
	2464	7.9	13.3	12.8
	2305	7.8	14.0	12.8
	2395	8.2		8.4
		9.4		7.7
		10.6		19.6
		NQ		22.6
		NQ		21.2
AVG	2388	8.6	13.4	14.7
STD	65.1	1.1	0.4	5.4
RSD	2.7	12.3	3.1	36.4
N	4	8	3.0	8

		% C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB2	173	21.1	18.1	16.7
	218	19.8	26.4	16.7
	273	20.3	30.8	16.7
	245	32.6		14.1
		20.3		14.1
		37.9		52.4
		33.0		65.2
		35.2		59.9
AVG	227	27.5	25.1	32.0
STD	36.9	7.3	5.3	21.3
RSD	16.2	26.6	21.0	66.7
N	4	8	3	8

CORN MEAL - FRYING - LEVEL 2

		% C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB1	10909	16.3	23.7	31.6
	10182	10.1	23.1	22.0
	8773	10.1	22.3	14.0
	4955	10.2	23.3	14.9
	9955	13.2	23.9	
		15.0		
		14.2		
		NQ		
AVG	8955	12.7	23.3	20.6
STD	2114.6	2.4	0.6	7.1
RSD	23.6	18.9	2.4	34.2
N	5	8	5	4

		% C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB2	2136	11.1	14.5	13.2
	2136	7.2	12.4	11.1
	2909	6.8	12.8	9.4
	2136	7.2	12.4	9.4
	2409	9.4	9.4	
		10.2		
		9.8		
		9.0		
AVG	2345	8.8	12.3	10.8
STD	301.1	1.5	1.6	1.6
RSD	12.8	16.9	13.4	14.5
N	5	8	5	4

C = FB1 or FB2 concentration (ng/g) of processed products
Co = FB1or FB2 concentration (ng/g) of unprocessed products
%C/Co = C /Co * 100
NQ= Not quantifiable

Table 2 RATIO OF OBTAINED CONCENTRATION / INITIAL CONCENTRATION (C/Co)

CORN - AUTOCLAVING - LEVEL 1

		%C/Co		
	Co	Batch 1(104*)	Batch 2 (108*)	Batch 3(97*)
FB1	578	39.7	12.4	72.9
	594	28.1	10.0	61.2
	586	14.0	12.4	67.0
	436			
AVG	549	27.3	11.6	67.0
STD	65.2	10.5	1.1	4.8
RSD	11.9	38.5	9.8	7.1
N	4	3	3	3

		%C/Co		
	Co	Batch 1(104*)	Batch 2(108*)	Batch 3(97*)
FB2	236	32.7	ND	96.9
	224	21.5	ND	91.5
	234	11.2	ND	93.7
	199			
AVG	223	21.8		94.0
STD	14.7	8.8		2.2
RSD	6.6	40.3		2.4
N	4	3	3	3

CORN - AUTOCLAVING - LEVEL 2

		%C/Co		
	Co	Batch 1(104*)	Batch 2(108*)	Batch 3(97*)
FB1	NQ	30.8	21.0	33.9
	2071	36.4	20.3	34.6
	2109	33.6	21.0	36.7
	2090			
AVG	2090	33.6	20.8	35.1
STD	15.5	2.3	0.3	1.2
RSD	0.7	6.8	1.6	3.4
N	4	3	3	3

		%C/Co		
	Co	Batch 1(104*)	Batch 2(108*)	Batch 3(97*)
FB2	236	26.3	ND	ND
	269	26.3	ND	ND
	259	26.3	ND	ND
	269			
AVG	258	26.3		
STD	13.5	0.0		
RSD	5.2	0.0		
N	4	3	3	3

C = FB1 or FB2 concentration (ng/g) of processed products

Co = FB1 or FB2 concentration (ng/g) of unprocessed products

%C/Co = C /Co * 100

NQ Not quantifiable

ND Not detected, detection limit = 8 ng/g

*Heat units

The number of heat units above 100°C for each run was determined and reported in Table 2, showing a relationship between elevated temperature and higher reduction. Based on studies of fumonisin degradation in maize fungal cultures, the extent of thermal degradation of FB1 and FB 2 was within the expected range compared to dry heat. The reductions obtained by autoclaving on canned maize of approximately 70% were greater than those obtained by Castelo et al (38). The difference might be related to our analyses of naturally contaminated maize instead of inoculated samples.

Extruded batter made from maize flour with the lower initial concentration of FB1 and FB2 yielded significant fumonisins reductions of approximately 70%, with %C/C0 ratios of 19.7 to 42.4 (Table 3). Analyses of replicate samples gave low deviations. No AP1 was detected in these samples. Based on studies of fumonisin degradation in maize fungal cultures, the extent of thermal degradation of FB1 and FB2 is close to twice the value using dry heat.

In contrast, extruded batter made from maize flour with the high initial concentration of FB1 and FB2 (Table 3) yielded very high (and statistically significant) reductions in fumonisins (approximately 90%). This was much higher than expected in comparison with the above data, with %C/C0 ratios as low as 6.2. Further studies are required to confirm this finding. Fumonisin levels on extruded products are comparable to those reported by Castelo et al (39)

Table 3 RATIO OF OBTAINED CONCENTRATION / INITIAL CONCENTRATION (C/Co)

CORN FLOUR - EXTRUSION - LEVEL 1

		%C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB1	523	35.8	34.6	30.2
	494	36.7	36.7	29.1
	552	36.7	36.7	32.5
AVG	523	36.4	36.0	30.6
STD	23.7	0.4	1.0	1.4
RSD	4.5	1.2	2.7	4.6
N	3	3	3	3

		%C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB2	66	21.2	43.9	21.2
	65	16.7	39.4	21.2
	67	21.2	43.9	21.2
AVG	66	19.7	42.4	21.2
STD	0.8	2.1	2.1	0.0
RSD	1.2	10.8	5.0	0.0
N	3	3	3	3

CORN FLOUR - EXTRUSION - LEVEL 2

		%C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB1	NQ	7.8	10.4	6.2
	1110	7.4	10.0	6.2
	1123	9.1	10.4	6.2
	1123	8.4		
AVG	1119	8.2	10.3	6.2
STD	6.1	0.6	0.2	0.0
RSD	0.5	7.9	1.8	0.0
N	4	4	3	3

		%C/Co		
	Co	Batch 1	Batch 2	Batch 3
FB2	180	ND	ND	ND
	205	ND	ND	ND
	205	ND	ND	ND
	205	ND		
AVG	199			
STD	10.8			
RSD	5.4			
N	4	4	3	3

C = FB1 or FB2 concentration (ng/g) of processed products
Co = FB1 or FB2 concentration (ng/g) of unprocessed products
%C/Co = C /Co * 100
NQ= Not quantifiable
ND Not detected, detection limit = 8 ng/g

Conclusions

It appears that *F. verticilloides* is ubiquitous in maize kernels and is possibly endophytic in character (4). This suggests that some concentrations of fumonisin are inevitable in the maize crop, particularly in warm areas. The effective management of fumonisin contamination includes good knowledge of the impact of industrial food processing practices. Our results suggest that when maize foods are heated at temperature and pressure as in frying and extrusion, considerable reductions in FB1 and FB 2 take place without apparent conversion into AP1. The data confirm reports of the more modest effect of autoclaving and higher reductions in processing systems employing elevated temperature and pressure. The identities of thermal breakdown products of fumonisin and their toxicities merits further study.

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