

# Effect of moisture content, lactic acid addition and extrusion conditions on reduction of $\beta$ -aflatoxins in milled sorghum (*Sorghum L. Moench*)

**A. Méndez-Albores<sup>1,4</sup>, F. Martínez-Bustos<sup>2</sup>, J. Véles-Medina<sup>2</sup>,  
E. Castaño-Tostado<sup>3</sup> and E. Moreno-Martínez<sup>1</sup>**

<sup>1</sup>UNAM -FESC. Av. J. Jiménez Cantú S/N, Col. Atlámica  
Cuautitlán Izcalli, C.P. 57740, México

<sup>2</sup>CINVESTAV-IPN

Apartado postal 1-798, C. P. 76230, Querétaro-México

<sup>3</sup>DIPA-Autonomous University of Queretaro  
Cerro de las Campanas, S/N C.P. 76010, Querétaro, México

(Received 11 June 2007; revised version 16 May 2008; accepted 24 June 2008)

## ABSTRACT

Sorghum flour contaminated with  $\beta$ -aflatoxins at a level of  $140 \pm 7.34$  ng/g was extrusion-cooked in a single screw extruder under two different conditions: moderate (ME) and severe (SE). The difference in the processing conditions was in the temperature profile of the barrel sections of the extruder: 60-80-100°C for ME and 80-150-200°C for SE, respectively. The flour moisture content (MC) was adjusted at 20, 25 and 30% by means of aqueous lactic acid (LA) at concentrations of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 N. The effect of the three extrusion variables (temperature profile, MC and LA concentration) was analysed as a completely randomized factorial design  $2 \times 3 \times 6$ . The barrel temperature profile, in combination with the MC and LA concentration, significantly affected the extent of aflatoxin reduction in the extruded sorghum. The recovered aflatoxin decreased with an increase in MC and LA concentration, in both temperature-profiles evaluated. The SE condition produced higher aflatoxin degradation rates (from 16.38 to 67.09%) than the ME condition (up to 19.79%).

KEY WORDS: sorghum,  $\beta$ -aflatoxins, lactic acid, extrusion-cooking, detoxification

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<sup>4</sup> Corresponding author: e-mail: albores@walla.com

## INTRODUCTION

Contamination of agricultural commodities with mycotoxins presents a serious hazard for human and animal health. Many countries have, therefore, established measures to control the contamination by mycotoxins of food and feedstuffs (EU, 2006).

The enforcement of legal limits for mycotoxins in animal feed is important for the health protection of potential consumer of any edible animal product which may be contaminated with mycotoxins, as well as for protection against economical losses due to the adverse effects exerted by some mycotoxins on animal productivity, such as in the production of meat, milk and eggs.

The Food and Agriculture Organization (FAO) estimates that at least 25% of world cereal production is contaminated with mycotoxins (Dowling, 1997). For this reason, development of detoxification procedures are needed. Such detoxification procedures should not only reduce the concentration of toxins to “safe” levels (below regulatory limits), but also prevent production of toxic degradation products and non-reduction of the nutritional value of the treated commodities. A number of approaches have been tried in an attempt to detoxify aflatoxins; however, only a few have yielded practical applications. Among these, ammoniation has proven to be an effective and cost-efficient means for reducing the aflatoxin content in peanut meal, maize and cottonseed destined for animal feeding (Marth and Doyle, 1979).

Reductions in mycotoxin levels, microbial contamination, and other toxic substances (such as trypsin inhibitors) are achieved during the cooking, particularly in the extrusion-cooking (Cazzaniga et al., 2001; Buser and Abbas, 2002; Castells et al., 2005). High temperature/short time extrusion-cooking methods are used in the industry to produce expanded products such as snack foods, breakfast cereals and pet foods (Rahman, 1995). Results for these methods have proven to be favourable for detoxification.

Also, organic acids have shown in previous studies to perform a detoxification function in treating aflatoxin-contaminated food/feeds (Méndez-Albores et al., 2005, 2007). These results suggest that detoxification of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) initially involves the formation of the  $\beta$ -keto acid structure (catalysed by the acidic medium), followed by hydrolysis of the lactone ring yielding aflatoxin D<sub>1</sub> (AFD<sub>1</sub>), derived from decarboxylation of the lactone ring-opened form of AFB<sub>1</sub>, which is 450 times less mutagenic than AFB<sub>1</sub> and presents an 18-fold toxicity decrease. Since no information has been available concerning the use of lactic acid during the extrusion-cooking of aflatoxin-contaminated sorghum, the purpose of the present study was to determine the effect of moisture content, addition of lactic acid, and various processing conditions (temperature profiles) on the stability

of  $\beta$ -aflatoxins, in an attempt to validate practical detoxification procedures for large-scale application.

## EXPERIMENTAL

Experimental units (EU) of sorghum flour contaminated with  $\beta$ -aflatoxins ( $140 \pm 7.34$  ng/g) were extrusion-cooked under two temperature profiles, three different moisture contents (MC), and six lactic acid (LA) concentrations. The effect of these extrusion variables was analysed as a completely randomized factorial design  $2 \times 3 \times 6$  with two replicates. The first factor corresponded to the extrusion-cooking conditions (ME and SE), the second to the sample MC (20, 25 and 30%), and the third to the aqueous LA concentration (0, 0.5, 1.0, 2.0, 4.0 and 8.0 N).

Chemicals such as  $\beta$ -aflatoxins and LA (85% purity) were obtained from Sigma Chemical Co. Ltd. (St Louis, MO, USA). Other analytical chemicals were obtained from J.T. Baker Mexico (Plomo 2, Industrial Xalostoc, México).

Procedures used for handling contaminated AFB materials were adopted from recommendations published by the International Agency for Research on Cancer (Castegnaro et al., 1981)

Sorghum grain of the commercial variety RB-3030 was utilized, with initial MC of 9.7 and 65% of seeds invaded by *Fusarium verticillioides*. Moisture content was determined by drying replicate portions of 5-10 g each of whole grain at 103°C for 72 h, with percentages calculated on a wet-weight basis. The grain was aflatoxin-free, as tested with the AOAC (1995) immunoaffinity column method described below.

The fungus *Aspergillus flavus* originally isolated from aflatoxin-contaminated maize, UNIGRAS-1231 (Culture Collection of the Grain and Seed Research Unit of the National Autonomous University of Mexico) was inoculated into Petri dishes containing MSA medium (%: malt extract, 2; sodium chloride, 6; and agar, 2) for 7 days at 25°C. This strain UNIGRAS-1231 is only capable of producing AFB<sub>2</sub> and AFB<sub>1</sub>. In order to inoculate the sorghum grain, the fungal spores were removed from the Petri dishes with a spatula; a sterile-water spore suspension (5.8 l) was prepared with approximately 100,000 conidia/ml, and this suspension was used to elevate the MC of the grain. This amount of inoculum ( $\approx 10,500$  spores/g of sorghum) was determined in order to eliminate competition with other storage fungi which could potentially grow under such incubation moisture and temperature conditions.

The total amount of sorghum to be inoculated was 55 kg. The MC of the grain was adjusted to 18% and stored in plastic bottles (5 kg of sorghum per replicate). Bottles were covered with thin polyethylene film to minimize the loss of humidity

from the grain. However, ten perforations with a pin were made to each film to avoid the accumulation of carbon dioxide generated by the respiration of grains and fungi. Bottles were incubated at 27°C during 24 d. After the incubation period, the grain was put under a 1000 mg/l ethylene oxide gas atmosphere for 5 h, to stop further development of the toxigenic fungus and to avoid the dispersal of viable spores. Finally, the aflatoxin-contaminated grain was finely ground in a mill (Pulvex-200, Pulvex, S.A. de C.V. Plutarco Calles 290, Mexico, DF), dried to 11.5% MC, and transferred to clean plastic bags, labeled, and stored at 4°C to await aflatoxin analysis.

The aflatoxin content was determined according to the 991.31 AOAC (1995) method, using monoclonal antibody columns for aflatoxins B<sub>2</sub> and B<sub>1</sub> (VICAM Science Technology, 303 Pleasant St., Watertown, MA, USA). When the concentration of total aflatoxins was greater than 25 ng/g, dilutions from the extract were made for their quantification in the fluorometer after reaction with a bromine solution at 0.002% (Candlish et al., 1991). The detection limit for aflatoxins with the immunoaffinity column (IAC) *via* fluorescence measurement is approximately 0.5 ng/g (Hansen, 1990). Aflatoxin identification was carried out by means of a Waters HPLC equipment with two pumps (Mod 510. Waters Associates, Milford, MA) and a Waters Nova-Pak C18, reverse phase column (5 µm, 3.9 × 150 mm). Standards as well as samples collected from the IAC (20 µl), were injected into an HPLC and eluted isocratically with a mobile phase of 12.5 mN acetic acid:acetonitrile (1:1, v/v) at a flow rate of 1 ml/min. Aflatoxins were fluorometrically detected and identified using a fluorescence detector Waters 470AC; the excitation and emission wavelengths were 338 and 425 nm, respectively. Aflatoxins were identified by their retention time (Rt), and compared with those for a pure aflatoxin standard solution under identical conditions. The performance of the 991.31 AOAC (1995) method was tested by the percentage of aflatoxin recovery by means of the HPLC method.

The MC of each EU consisting of 700 g milled sorghum was adjusted to 20, 25 or 30% by adding 78.1, 132.3 or 194.2 ml of aqueous LA solution at 0, 0.5, 1.0, 2.0, 4.0 or 8.0 N (the range of LA expressed in percentage was from 0.5 to 20%). Samples were mixed at low speed for 15 min in a mixer (model C-100, Hobart Corp., Troy, OH). After mixing, samples were transferred to plastic bags and stored at 4°C for 72 h, in order to achieve MC equilibration (after which period, MC was determined). The total number of EU was 72 (36 for each extrusion-cooking condition). The pH of the milled sorghum and extrudates was determined according to the 02-52 AACC method (2000).

A laboratory scale single screw extruder was used, designed and manufactured by CINVESTAV-IPN, Mexico with a barrel diameter of 2.5 cm, length of 42.8 cm, screw compression ratio of 1:1, and a 3-mm diameter cylindrical die. The barrel was equipped with electrical cartridge heaters and three independently controlled heating

and cooling zones. The feed rate of 16 rpm (73 g/min) and the screw speed of 30 rpm were constant throughout the experiment. The difference in the processing conditions was in the temperature profile in the barrel sections: 60-80-100°C for the ME condition, and 80-150-200°C for the SE condition. The range of temperature fluctuation in each zone was approximately  $\pm 2^\circ\text{C}$ . The extruder was not stopped or cleaned between samples; therefore, a portion of the next test material was used to purge the extruder. Each EU was run through the extruder, and approximately 500 g of extrudate sample was collected after achieving steady-state flow in the extruder. Extruded samples were oven-dried at 40°C for 24 h and stored at 4°C until analysed.

Analysis of variance (ANOVA) was used to evaluate the effect of temperature profile, moisture content and lactic acid concentration. Means were separated by the Tukey procedure using the Statistical Analysis System (SAS, 1998).

## RESULTS AND DISCUSSION

The total  $\beta$ -aflatoxins concentration in the inoculated sorghum was  $140 \pm 7.34$  ng/g. This aflatoxin concentration is not commonly found in the commercial sorghum used to produce foods or feeds. The idea was to use it to test the potential detoxifying activity of LA under these specific conditions of extrusion. The chromatograms of the HPLC (not presented) indicated that the toxins in the sorghum flour were AFB<sub>2</sub> and AFB<sub>1</sub>, with concentrations of 11.2 and 128.8 ng/g, respectively. It has been stated that *A. flavus* produces mainly  $\beta$ -toxins (De Arriola et al., 1988). The HPLC chromatograms, however, indicate that clear separation of aflatoxins occurred; the Rt values for AFB<sub>2</sub> and AFB<sub>1</sub> standards were 3.7 and 4.0 min, respectively.

Table 1 shows the MC of the sorghum flour before extrusion-cooking:  $19.89 \pm 0.05$ ,  $25.12 \pm 0.08$ ,  $30.29 \pm 0.07\%$  for samples to be processed with ME conditions, and  $20.39 \pm 0.17$ ,  $25.30 \pm 0.22$ ,  $30.30 \pm 0.12\%$  for samples to be processed with SE conditions. Consequently, the MC of all EU was considered to have been stable throughout the equilibrating period (72 h at 4°C).

In general, extrusion caused MC reductions around  $29.94 \pm 4.07\%$  and  $67.50 \pm 2.68\%$ , in samples processed under conditions ME and SE, respectively (Table 1). These reductions in MC are attributable to the differences in temperature profiles under both extrusion conditions. Statistical differences in pH (Table 1) were not observed before or after the extrusion-cooking of the samples under both conditions evaluated. The pH levels of the adjusted sorghum flour (data not presented) were essentially the same as those registered for extrudates. As LA concentration and MC increased, lower pH values were observed. Samples with no acid (control) presented an average pH value of 6.10; while the lowest pH value of near 3.46 was observed in samples processed with 30% MC and 8.0 N LA (Table 1).

Table 1. Moisture content and degree of acidity (pH) of milled sorghum and extrudates

Treatment	LA		Flour MC, %		Extrudates MC, %		Extrudates pH	
	N	SE	ME	SE	ME	SE	ME	SE
Adjusted to 20%	0.0	19.87 ± 0.45 <sup>a</sup>	20.29 ± 0.51 <sup>a</sup>	14.96 ± 0.09 <sup>a</sup>	7.26 ± 0.03 <sup>a</sup>	6.13 ± 0.5 <sup>3a</sup>	6.10 ± 0.7 <sup>3a</sup>	
	0.5	19.93 ± 0.08 <sup>a</sup>	20.36 ± 0.51 <sup>a</sup>	14.66 ± 0.08 <sup>a</sup>	7.17 ± 0.07 <sup>a</sup>	5.73 ± 0.5 <sup>3b</sup>	5.72 ± 0.5 <sup>3b</sup>	
	1.0	19.96 ± 0.13 <sup>a</sup>	20.47 ± 0.09 <sup>a</sup>	14.70 ± 0.19 <sup>a</sup>	7.27 ± 0.04 <sup>a</sup>	5.45 ± 0.6 <sup>3c</sup>	5.45 ± 0.6 <sup>3c</sup>	
	2.0	19.89 ± 0.09 <sup>a</sup>	20.58 ± 0.50 <sup>a</sup>	14.43 ± 0.24 <sup>a</sup>	7.22 ± 0.05 <sup>a</sup>	5.11 ± 0.4 <sup>3d</sup>	5.11 ± 0.4 <sup>3d</sup>	
	4.0	19.85 ± 0.14 <sup>a</sup>	20.12 ± 0.06 <sup>a</sup>	14.41 ± 0.18 <sup>a</sup>	7.18 ± 0.07 <sup>a</sup>	4.65 ± 0.5 <sup>3e</sup>	4.65 ± 0.4 <sup>3e</sup>	
	8.0	19.86 ± 0.15 <sup>a</sup>	20.51 ± 0.30 <sup>a</sup>	14.35 ± 0.05 <sup>a</sup>	7.28 ± 0.04 <sup>a</sup>	4.08 ± 1.1 <sup>3f</sup>	4.08 ± 0.5 <sup>3f</sup>	
Adjusted to 25%	0.0	25.01 ± 0.18 <sup>a</sup>	25.36 ± 0.28 <sup>a</sup>	17.66 ± 0.30 <sup>a</sup>	7.79 ± 0.03 <sup>a</sup>	6.11 ± 0.5 <sup>3a</sup>	6.11 ± 1.0 <sup>3a</sup>	
	0.5	25.12 ± 0.27 <sup>a</sup>	25.15 ± 0.13 <sup>a</sup>	17.83 ± 0.36 <sup>a</sup>	7.57 ± 0.19 <sup>a</sup>	5.62 ± 0.6 <sup>3b</sup>	5.65 ± 0.6 <sup>3b</sup>	
	1.0	25.03 ± 0.11 <sup>a</sup>	25.40 ± 0.47 <sup>a</sup>	17.97 ± 0.14 <sup>a</sup>	7.58 ± 0.04 <sup>a</sup>	5.32 ± 1.0 <sup>3c</sup>	5.33 ± 0.4 <sup>3c</sup>	
	2.0	25.17 ± 0.04 <sup>a</sup>	25.12 ± 0.11 <sup>a</sup>	18.10 ± 0.06 <sup>a</sup>	7.61 ± 0.03 <sup>a</sup>	4.89 ± 0.5 <sup>3d</sup>	4.92 ± 0.5 <sup>3d</sup>	
	4.0	25.20 ± 0.06 <sup>a</sup>	25.10 ± 0.17 <sup>a</sup>	18.00 ± 0.01 <sup>a</sup>	7.69 ± 0.11 <sup>a</sup>	4.27 ± 0.4 <sup>3e</sup>	4.29 ± 0.4 <sup>3e</sup>	
	8.0	25.14 ± 0.05 <sup>a</sup>	25.68 ± 0.49 <sup>a</sup>	18.01 ± 0.01 <sup>a</sup>	7.66 ± 0.16 <sup>a</sup>	3.59 ± 0.4 <sup>3f</sup>	3.61 ± 0.5 <sup>3f</sup>	
Adjusted to 30%	0.0	30.38 ± 0.23 <sup>a</sup>	30.17 ± 0.08 <sup>a</sup>	19.70 ± 0.31 <sup>a</sup>	9.71 ± 0.27 <sup>a</sup>	6.11 ± 2.5 <sup>3a</sup>	6.06 ± 1.5 <sup>3a</sup>	
	0.5	30.24 ± 0.21 <sup>a</sup>	30.35 ± 0.01 <sup>a</sup>	19.62 ± 0.48 <sup>a</sup>	9.59 ± 0.20 <sup>a</sup>	5.54 ± 0.5 <sup>3b</sup>	5.53 ± 0.3 <sup>3b</sup>	
	1.0	30.26 ± 0.13 <sup>a</sup>	30.48 ± 0.31 <sup>a</sup>	19.70 ± 0.26 <sup>a</sup>	9.60 ± 0.07 <sup>a</sup>	5.17 ± 1.2 <sup>3c</sup>	5.20 ± 0.7 <sup>3c</sup>	
	2.0	30.33 ± 0.29 <sup>a</sup>	30.37 ± 0.13 <sup>a</sup>	19.93 ± 0.11 <sup>a</sup>	9.56 ± 0.09 <sup>a</sup>	4.71 ± 1.1 <sup>3d</sup>	4.80 ± 0.5 <sup>3d</sup>	
	4.0	30.31 ± 0.05 <sup>a</sup>	30.18 ± 0.09 <sup>a</sup>	20.02 ± 0.16 <sup>a</sup>	9.62 ± 0.05 <sup>a</sup>	4.07 ± 0.5 <sup>3e</sup>	4.09 ± 0.4 <sup>3e</sup>	
	8.0	30.20 ± 0.10 <sup>a</sup>	30.26 ± 0.03 <sup>a</sup>	20.06 ± 0.05 <sup>a</sup>	9.76 ± 0.19 <sup>a</sup>	3.46 ± 0.6 <sup>3f</sup>	3.47 ± 0.8 <sup>3f</sup>	

LA - lactic acid; ME - moderate extrusion condition; SE - severe extrusion condition; MC - moisture content mean of three replicates ± standard deviation for each MC, means with same letter in the same column are not significantly different (Tukey<0.05)

Different levels of LA (50 and 100 g/kg dry matter) added to silage for dairy cows (a mixture of barley and soyabean meal, 75:25) were investigated to evaluate the effect of LA on silage intake and milk production, with and without a post-ruminal supplement of 230 g/d sodium caseinate (Choung and Chamberlain, 1993). Both levels of LA added ultimately reduced the intake of silage, and milk yield was reduced only with the addition of 100 g LA; however, this effect was reversed when casein was administered post-rationally. In all treatments, casein infusion increased the yield of protein in milk by 30-35 g/d, and protein concentration in milk was also increased linearly with the addition of LA to the silage. In this research, it is possible that similar beneficial as well as detrimental effects occur in relation to high doses of LA added to sorghum. In relation to those doses of LA used in the present research, they were in the range of 0.5 to 20%. Doses higher than 10% should be tested for possible detrimental effects in feed for different species of animals. Dilution of acid treated raw materials could be another way to reduce the noxious effects of these ingredients in feed.

The stability of  $\beta$ -aflatoxins in the sorghum was significantly affected by the extrusion-cooking parameters (temperature profile, sample MC, and LA concentration) in both conditions evaluated (Figure 1). The amount of  $\beta$ -aflatoxins decreased, as MC and LA concentration increased. The highest reduction in the aflatoxin content (67.09%) was observed in the case of extrudates obtained under conditions of SE at 30% MC and 8.0 N LA. In these samples, the final aflatoxin content was  $46.06 \pm 1.22$  ng/g (Figure 1, profile a). However, only a 19.79% aflatoxin reduction occurred during the extrusion-cooking by means of ME conditions in samples extruded with 30% MC and 8.0 N LA; these samples, had a final aflatoxin content of  $112.33 \pm 1.97$  ng/g (Figure 1, profile b).

In samples without the addition of LA (controls) and processed under ME conditions, there was no apparent reduction in the aflatoxin content; however, when controls were processed under conditions of SE, a 24.95% reduction in the aflatoxin content was observed (Figure 1). This reduction may possibly be due to the combined action of MC and a higher temperature profile in SE condition. Under these conditions (SE), sorghum was processed with high temperature/pressure combined with severe mechanical shear stress, which resulted in a moderate reduction in the aflatoxin content without additive addition (LA). It is well known that aflatoxins are relatively heat-stable and are not completely destroyed when thermally treated to produce food or feeds.  $\beta$ -Toxins have been found to be unstable up to their melting points of around 250°C (Feuell, 1966).

In this type of experimental extruder, the product resides in the barrel for 59 sec, with consequent moderate reductions of  $\beta$ -aflatoxins observed. Previous studies indicate that higher temperatures and longer times are required to significantly reduce the levels of aflatoxins during food processing (Samarajeewa et al., 1990).



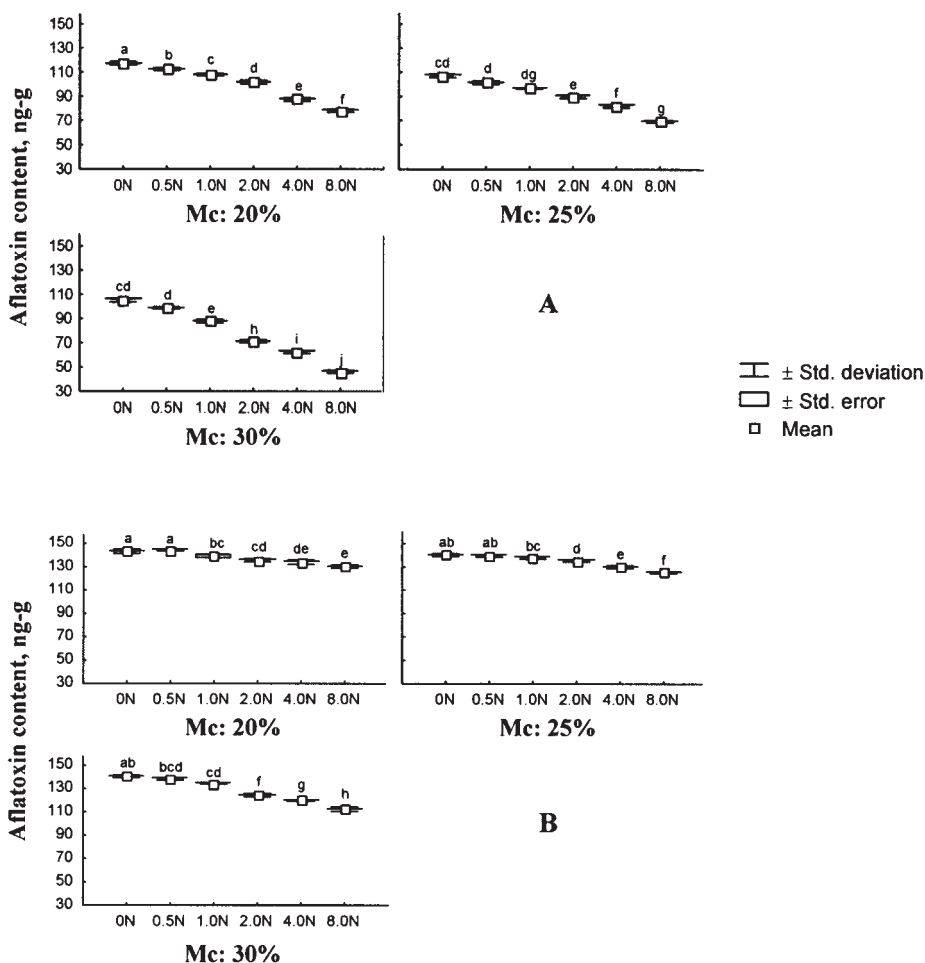


Figure 1. Lactic acid effect on aflatoxin reduction during extrusion of contaminated sorghum with different temperature profiles: A - severe, B - moderate  
 \* for each temperature profile, box and whiskers with same letter are not significantly different (Tukey<0.05)

Castells et al. (2005) reported that the extrusion of rice flour contaminated with AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>, reduced the levels by 51 to 95%, depending on the type of aflatoxin and variables studied such as MC, barrel temperature, and residence time. The same authors reported reductions of 100, 95, and 83% for fumonisins, aflatoxins, and zearalenone, during extrusion-cooking of cereals, and lower reductions for deoxynivalenol, ochratoxin A and moniliformin, where toxin reductions did not exceed 55, 40 and 30%, respectively.



Saalia and Phillips (2002) reported that the extent of aflatoxin detoxification during the extrusion of yellow maize with high levels of spiked aflatoxins (150 ng/g for AFB<sub>1</sub> and AFG<sub>1</sub>, and 45 ng/g for AFB<sub>2</sub> and AFG<sub>2</sub>) was dependent on the temperature, MC, pH, and nucleophile addition. With a residence time of 85 sec, the best conditions for aflatoxin reduction were treatments at 35% MC and pH 9, with or without lysine addition. Under these conditions, 62% reduction in the content of total aflatoxins was achieved. The same authors reported that pH showed significant aflatoxin reduction, even without heating. Cazzaniga et al. (2001) reported that extrusion of maize flour at low levels of AFB<sub>1</sub> (50 ng/g), was partially successful (10-25%) for the decontamination with metabisulphite addition (1%) at temperatures of 150 and 180°C, respectively. In the case of aflatoxin contaminated cottonseed (339 ng/g AFB<sub>1</sub>), reductions of 46 and 65% were observed, when samples were extrusion-cooked at 104 and 160°C, respectively. Furthermore, multiple-pass extrusion produced an additional 55% of aflatoxin reduction when cottonseed was extruded four times as compared to one time (Buser and Abbas, 2002).

In relation to the above cited aflatoxin reductions, it is noted that the detoxification of contaminated materials varies considerably, depending on the extrusion processing parameters, such as: screw configuration, feed moisture content, temperature profile in the barrel sections, screw speed, feed rate, final die configuration, initial aflatoxin concentration and the use of additives. It is important to note that the type of extruder also could have a significant impact on the residence time, the degree of mixing and internal pressure and that this factor could alter the performance of the equipment used to detoxify the extruded materials.

## CONCLUSIONS

Extrusion-cooking temperature, moisture content (MC) as well as lactic acid (LA) concentration, significantly affected the stability of  $\beta$ -aflatoxins in the milled sorghum. Lower levels of  $\beta$ -aflatoxins (46.06 ng/g) were found in samples processed with the higher temperature profile, coupled with the highest MC and LA concentration. Assuming that similar extrusion-cooking conditions could be used for the production of feeds, reductions of about 67.09% in  $\beta$ -aflatoxins levels can be expected. The results suggest that more severe extrusion conditions with respect to these three variables (temperature, MC, and LA concentration) are required to obtain higher detoxification rates of  $\beta$ -aflatoxins in the contaminated sorghum.

## ACKNOWLEDGEMENTS

The authors are grateful to DGAPA/UNAM for the first author postdoctoral fellowship. We wish to thank Elisabeth Buchanan for her English revision.

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