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Decontamination of aflatoxin-contaminated maize by dehulling

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Abstract: Dehulling of maize grains as an aflatoxin decontamination method was investigated. Sixty kilograms of maize (whose average moisture content was 110 g kg^{-1}) were thoroughly mixed and divided into two samples. The kernel moisture content of one sample was adjusted to 200 g kg^{-1} while the other (control) was left at 110 g kg^{-1}. The two samples were kept at ambient temperature (25–30 °C) for 21 days. Twenty sub-samples, ten from each, were further divided into two so that one lot was dehulled while the other was not dehulled. These sub-samples were then milled to pass through a 1 mm screen and the meal was evaluated for aflatoxin contamination. It was found that there was a 92% decrease in aflatoxin levels in dehulled maize meal compared with undehulled maize meal. We therefore suggest that dehulling the grain can be used to reduce aflatoxin levels in maize.

Keywords: aflatoxin; decontamination; dehulling; maize

INTRODUCTION

Aflatoxins are highly toxic and carcinogenic metabolites produced by fungi of the *Aspergillus* genus. They are a serious problem in many countries of the world, especially developing countries. They occur in almost all agricultural produce. A lot of effort, resources and time have been committed to aflatoxin research work since the early 1960s. Consequently, a substantial amount of knowledge has been generated concerning the conditions which predispose grain to aflatoxin contamination, whether in the field or in storage. An equal effort and attention has also been given to possible methods that could be used to prevent contamination of grain by aflatoxin-producing fungi. These include use and/or evaluation of aflatoxin-resistant varieties of seeds,[1] minimizing excess water and/or stress to crops during their various growing stages, good timing of harvest, proper harvesting techniques which minimize damage to grain as well as effecting proper storage conditions of temperature, moisture, pH and gas atmosphere.[2]

For stored grain lots, approaches to detoxification have included physical methods, eg gamma irradiation of grain and electronic sorting.[3,4] Segregation of kernels by particle size has also been used. It was shown that larger grains (>4.5 mm) contained significantly lower aflatoxin levels than those smaller than 4.5 mm.[5] Heat treatment such as cooking[6] and dry roasting[7,8] have been reported to reduce aflatoxin concentration. Chemical treatment of grain by hydrogen peroxide,[9] sodium hypochlorite,[10] ozone,[11] sodium bisulfate[12] and aqueous or gaseous ammonia with or without heating[13] has been used to decontaminate grain and oil seeds. Biological methods have also been used to degrade or remove aflatoxin from ground samples of maize and peanuts. Species of bacteria such as *Flavobacterium aurantiacum*[14] and lactic acid bacteria[15,16] have been employed.

One of the early practices of African food preparation was manual dehulling of grain before grinding. This practice is no longer in common use except in a few countries,[17] and has been replaced by use of mechanical disc dehullers on a commercial and semi-commercial scale. Dehulling is a milling technique that removes the outer layers of grain by abrasion. As these layers are the regions most susceptible to fungal attack and aflatoxin accumulation,[18] we sought to find out the effect of dehulling maize contaminated with aflatoxins.

EXPERIMENTAL

Sixty kilograms of maize from the 1999/2000 season (moisture content of 110 g kg^{-1}) were obtained from

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the market and subdivided into two 30 kg samples after thorough mixing. One of the samples had its moisture content adjusted to 200 g kg^{-1} to allow colonization by the natural mycoflora of the maize[19] while the other sample (control) was left at 110 g kg^{-1}. After this treatment, the two lots were further subdivided into ten sub-samples each and kept in containers at ambient temperatures (25–30 °C) for 21 days to let fungi grow and produce mycotoxins. The containers were covered with damp jute bags, whose ends were immersed in bowls filled with water, to minimize evaporation and keep the relative humidity high and constant within the sample environment. The relative humidity and temperature were monitored using a Sunbeam hygrometer and thermometer (Sunbeam-Oster Household Products, Laurel, Mississippi, USA) over the period of experimentation. We made sure that the bags were not in direct contact with the samples at any time. Samples were then dried to about 110 g kg^{-1} moisture content. Five sub-samples from each treatment were ground to pass through a 1 mm mesh[20] whilst the other five sub-samples from each treatment were dehulled using a dehuller obtained from Rural Industries Innovation Centre, Kanye, Botswana, and the grains similarly ground. The bran and grits were also collected. The samples were then assayed for aflatoxins using HPLC as described by Siwela.[21] In brief, the method involved the use of aqueous acetone to extract the ground sample. An aliquot of the extract was mixed with lead acetate to precipitate proteins and other materials and then passed through a phenyl-bonded pre-packed column (Bond Elut, Varian, Harbor City, USA). The aflatoxins were then eluted using chloroform. The chloroform was evaporated and the residue derivatized using trifluoroacetic acid (TFA). The TFA was then evaporated and the residue reconstituted in 250 g kg^{-1} acetonitrile. Analyses were performed using a Philips high pressure liquid chromatograph (PU 4100M; Cambridge, UK) with manual injections of 25 μL aliquots of sample alongside standard solutions. Aqueous acetonitrile (250 g kg^{-1}) at a flow rate of 16.67 μL s^{-1} was used as the mobile phase. The toxins were detected using a Philips PU 4027 fluorescent detector set at wavelength 365 nm for excitation and 418 nm for emission; the signals were recorded on an electronic integrator (Varian 4400; Walnut Creek, USA). The concentrations of aflatoxins were calculated by comparing peak heights with those of standard solutions.

Statistical analysis

Results were expressed as means ± standard deviation (SD). Statistical analysis (Student's *t* test and Welch's *t* test) was performed using GraphPad Instat software (Graph Pad Prism, San Diego, California, USA).

RESULTS AND DISCUSSION

At the end of 21 days, the maize was determined to contain 198.70 ± 0.22 g kg^{-1} moisture. This represented a statistically non-significant change of 6.5 g kg^{-1} moisture (Welch's t test, $P < 0.05$). The relative humidity in the containers remained constant at 98% ± 2% and the mean temperature also remained constant at 22 °C. Despite observed fungal growth, no gross deterioration of the maize was evident. The grain looked and felt sound. Grain that has suffered gross deterioration as a result of fungal invasion is usually lighter in mass, discoloured, darkened[22] and friable. No attempt to systematically identify the fungi was made as the main aim of this study was to assay for aflatoxin contamination in the maize.

Table 1 shows aflatoxin levels in maize before and after dehulling as well as levels of aflatoxins after the moisture levels of maize were elevated from 110 to 200 g kg^{-1}. The high levels of aflatoxin in undehulled dried maize (control) can be attributed to unusually high rainfall and high temperatures towards the end of the 1999/2000 season due to cyclone Elina that affected Zimbabwe. Late season rainfall and cyclones have been shown to contribute significantly to aflatoxin content in maize left standing in the field.[23,24] The action limit for total aflatoxins is 20 μg kg^{-1} in Zimbabwe as defined by the Food Standards Act of 1971 revised in the Statutory Instrument of 1990.

Elevation of moisture content of the maize from 110 to 200 g kg^{-1} and keeping the grain at 25–30 °C for 21 days led to fungal growth with concomitant production of aflatoxins. The highest increases in aflatoxins were a 19-fold increase of aflatoxin B_1 and

Table 1. Aflatoxin levels[a] (μg kg^{-1} dry matter basis) in maize before and after dehulling

	Maize as received			Maize after moisture elevation		
Mycotoxin	Undehulled[b]	Dehulled[b]	Aflatoxin reduction (%)	Undehulled[c]	Dehulled[c]	Aflatoxin reduction (%)
Aflatoxin B_1	3.39 ± 0.75	0.38 ± 0.06	88.8	65.90 ± 16.43	6.70 ± 4.43	89.8
Aflatoxin G_1	15.12 ± 3.60	0.96 ± 0.19	93.7	93.30 ± 17.90	6.93 ± 2.28	92.6
Aflatoxin B_2	5.19 ± 0.68	0.47 ± 0.08	90.9	22.65 ± 10.10	1.92 ± 0.77	91.5
Aflatoxin G_2	42.20 ± 1.24	2.67 ± 0.25	93.7	43.19 ± 3.38	3.82 ± 1.01	91.2
Total AFT	67.42	4.48	93.4	225.04	19.37	91.4

[a] Data represent means and standard deviations for $n = 5$.
[bc] Differences in aflatoxin levels between undehulled and dehulled maize significantly different from each other (Student's *t* test, $P < 0.001$).

Table 2. Distribution ratios of aflatoxins in grits and bran before and after moisture treatment

	Maize as received		Maize after moisture elevation	
Mycotoxin	Grits	Bran	Grits	Bran
Aflatoxin B_1	1	28	1	24
Aflatoxin G_1	1	26	1	21
Aflatoxin B_2	1	13	1	12
Aflatoxin G_2	1	17	1	12

a six-fold increase of aflatoxin G_1 in the maize whose moisture levels were artificially elevated compared with controls. No significant changes in aflatoxin G_2 levels were noted while the levels of aflatoxin B_2 increased four-fold.

At the end of the incubation period (21 days) and subsequent drying, assay for aflatoxin in the maize showed a significant reduction of aflatoxin levels in dehulled maize compared with controls (Table 1). Dehulling effected an average of 92% reduction in aflatoxin levels. This figure relates to a level of dehulling resulting in 20% loss in weight of the grain due to the abrasion process. The presence of 92% of the total aflatoxin in the 20% residue obtained as a result of dehulling can be explained by the fact that although fungi are capable of penetration into the grains, surface colonization and hence aflatoxin production is more prevalent than internal infection.[19] The presence of aflatoxins in the dehulled maize indicates that the fungal mycelia do also penetrate the endosperm. This is in agreement with laboratory studies that have shown that *Aspergillus flavus* growth is distributed according to germ > tip cap > endosperm.[25]

The bran fraction of both the control maize kernels and those kernels whose moisture levels had been elevated contained the highest levels of aflatoxins (Table 2), while grits contained lower levels. Our results are in agreement with those of Trenholm *et al*[26] who reported high concentrations of deoxynivalenol and zearalenone toxin in bran of four dehulled cereals.

As cooking and steaming under pressure has been shown to reduce aflatoxins by up to 50%,[6] a combination of dehulling and cooking seems an attractive way of decontamination of maize-based foods and feed, particularly in Southern Africa where maize meal forms the staple food for humans. We therefore recommend dehulling of grains either manually or mechanically before preparation of food. Not only does dehulling remove aflatoxins from the outer layer of maize as we have shown in this report, but Hosney[27] has also stated that dehulling removes antinutritional factors such as phytate and reduces the lipid content of the seed, thus improving the storage quality of the resulting meal.

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