

Impact of mechanical shelling and dehulling on *Fusarium* infection and fumonisin contamination in maize

P. FANDOCHAN¹, R. AHOANSOU¹, P. HOUSSOU¹, K. HELL², W. F. O. MARASAS³, & M. J. WINGFIELD⁴

¹Programme on Agricultural and Food Technology, National Institute of Agricultural Research of Benin, Porto-Novo, Benin, ²International Institute of Tropical Agriculture (IITA), Cotonou, Benin, ³Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, Tygerberg, South Africa, and ⁴Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Biological and Agricultural Sciences University of Pretoria, Pretoria, South Africa

(Received 15 July 2005; accepted 28 October 2005)

Abstract

Mechanical shelling and dehulling methods were tested to evaluate their impact on *Fusarium* infection and fumonisin contamination in maize. All shelling methods which were tested were found to damage the grains. The IITA[®] sheller caused the highest level (up to 3.5%) of damage. *Fusarium* populations were higher on damaged grains, the highest being recorded from grains damaged by the IITA[®] sheller (2533.3 cfu g⁻¹). Fumonisin levels were higher in damaged grains, the highest being in maize shelled with the IITA[®] sheller (2.2 mg kg⁻¹). Fumonisin levels were positively and significantly correlated with the percentage of damage caused by the shelling methods, and with the number of *Fusarium* colonies in maize. Mechanical dehulling methods significantly reduced fumonisin levels in maize, resulting in a mean reduction of 62% for Mini-PRL, 65% for Engelberg, and 57% for the attrition disc mill. It is important for farmers to choose appropriate shelling methods to reduce mycotoxin contamination. Dehulling should be widely promoted for the reduction of mycotoxins in maize.

Keywords: Maize, impact, shelling, dehulling, *Fusarium*, fumonisins, contamination

Introduction

In Benin as in most West African countries, maize undergoes many postharvest operations before consumption, of which shelling and dehulling are of great importance. Shelling usually occurs prior to storage or processing and consists of separating grains from the maize cob's core. Dehulling consists of removing the pericarp from the grain. This is often accompanied by degerming (removal of the embryo).

Shelling and dehulling are generally carried out by women, and are very labour-intensive and time-consuming (Fandohan 2004). Shelling is traditionally done by hand, mortar and pestle or using a wooden stick (Houssou 2000), whereas dehulling is done by using stones or mortar and pestle (François 1988, Fandohan 2004). Generally, the output of manual shelling or dehulling is very low. Hand shelling maize from one hectare (approximately 1 tonne) by a single woman requires 16 days of labour

with an hourly output of 8–15 kg (FAO 1992). One woman can dehull approximately 10 kg of maize in one hour (François 1988, Fandohan 2004).

Different types of mechanical equipment are being introduced in rural and urban areas of Africa to make shelling and dehulling of maize easier, faster and more efficient. However, to date, little attention has been given to the possible effects these machines may have, not only on fungal infection but also on mycotoxin contamination of maize. Kozakiewicz (1996) stressed that postharvest mechanization in general, if not used correctly, can damage the processed products and may facilitate fungal infection.

The present study was undertaken to address this problem, targeting the impact of automated shelling and dehulling methods currently promoted in West Africa on *Fusarium* infection and fumonisin contamination of maize. Fumonisin are recently identified

mycotoxins produced by toxigenic *Fusarium* species such as *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushina) Nirenberg. These toxins have attracted increasing attention because of their adverse effects on animal and human health and their negative economic impact (Bolger et al. 2001). Fumonisin has been found to be associated with several animal diseases such as leukoencephalomalacia in horses (Kellerman et al. 1990) and pulmonary oedema in pigs (Harrison et al. 1990). Their occurrence in maize intended for human consumption has been linked to a higher incidence of oesophageal cancer (Rheeder et al. 1992, Chu & Li 1994) and liver cancer (Ueno et al. 1997).

Material and methods

Maize cultivar used

The 90-day cultivar DMR-ESR-W, an improved IITA variety, was used. DMR-ESR-W is known to be resistant to downy mildew (*Peronosclerospora sorghi*) and to maize streak virus (Schulthess et al. 2002).

Impact of different shelling methods on Fusarium and fumonisin contamination

Maize cobs, after harvest, were immediately dehusked and sun-dried to moisture content less than 18%. They were divided into four lots of at least 300 cobs each. The cobs of each lot were shelled using the following four methods with one shelling method for each lot. These methods included shelling by hand, shelling using a handle-operated sheller, and shelling using two motorized shellers type Renson® and type IITA®. Characteristics of these shellers are described in Table I.

After shelling, grains (10 kg) obtained from each lot were stored in jute bags in a room for three months. Each lot (shelling method) was replicated three times. A 500 g sample was taken from each bag at the beginning of the trial, and after one and three months of storage. This sample was used for determination of moisture content, percentage of damage caused by the shelling methods, *Fusarium* population and fumonisin levels.

Grain moisture content was determined just after sampling each bag using an electronic moisture meter (model HOH-EXPRESS HE 50, PFEUFFER, Germany). Percentage of grain damage caused by each shelling method was assessed after shelling at the beginning of the trial (Pantenius 1988). *Fusarium* species were enumerated using dilution plating at the beginning of the trial, and also at one and three months after stocking. Thus, 10 g of maize grains were finely ground, thoroughly

mixed with 90 ml of sterile 0.1% peptone water, and serial dilutions made to 10^{-2} . One millilitre of suspension was transferred into individual Petri dishes, mixed with potato dextrose agar (PDA) (15 ml) and the Petri dishes were incubated at 25°C for 5 days exposed to a 12:12-h light/dark regime. *Fusarium* colonies were isolated and transferred onto carnation leaf agar and incubated for seven days at 25°C exposed to 12:12-h light/dark regime. Colony forming units per gram of sample (cfu g^{-1}) were enumerated. *Fusarium* species were identified according to Nelson et al. (1983).

Fumonisin content was determined at the beginning of the trial, and after one and three months of storage using the VICAM method (VICAM 1998). Ground maize (50 g) was weighed into a flask and mixed with 5 g of sodium chloride and 100 ml of methanol:water (80:20). The mixture was homogenized at high speed for 1 min using a Waring blender (Waring Commercial, Torrington, USA), then filtered through a fluted filter paper. The extract (10 ml) was diluted with 40 ml of phosphate buffered saline (PBS)/0.1% Tween-20 wash buffer, and filtered through a 1.0 µm microfibre filter. The diluted extract was then passed through the immunoaffinity column (FumoniTest™ column, VICAM, Watertown, USA), which contains specific antibodies to fumonisins. The column was washed with 10 ml of PBS/0.1% Tween-20 wash buffer followed by 10 ml of PBS. Fumonisin was eluted from the column with 1 ml of HPLC grade methanol. A mixture of Developer A and Developer B (1 ml) was added to the eluate, and collected in a cuvette. Fumonisin levels were determined with a fluorometer (VICAM Fluorometer Series 4, Watertown, USA).

In order to reduce eventual influences of grain moisture content, insect damage and sheller speed on results during the study, the following precautions were observed. The cobs were sun-dried prior to shelling, to bring the grain moisture content to a level less than 18%. Prior to storage, the grains were dusted with the binary pesticide Sofagrain® (0.05% deltamethrin and 1.5% pirimiphos-methyl) to reduce insect damage. Visibly damaged and cracked grains were also carefully removed by hand. Efforts were made during the shelling operation to maintain the speed of the rotary cylinder inside the shelling chamber at 500 r/min.

Impact of different dehulling methods on Fusarium and fumonisin contamination

After three months of storage, grains from the bags of maize initially shelled with the two motorized shellers were thoroughly mixed and divided into three lots of approximately 7 kg each. Three replicates of 2 kg of maize were sampled from each lot and dehulled

Table I. Characteristics and use conditions of the different tested shelling and dehulling methods.

Characteristics	Shelling methods				Dehulling methods		
	Shelling by hand	Handle-operated sheller	Motorized sheller type Renson®	Motorized sheller type IITA®	Attrition disc mill	Engelberg	Mini-PRL
Manufacturer	–	Renson (France)	Renson (France)	IITA (Nigeria)	Amuda (India)	Rajan (India)	Père et Frère (Senegal)
Type of motor used	–	–	Honda (5 HP) (Petrol)	Briggs & Stratton (Petrol)	–	–	–
Mean speed of rotary cylinder (rpm)	–	–	500	500	–	–	–
Operation mode	–	–	–	–	Continuous	Continuous	Discontinuous
Principle	Friction	Friction	Friction	Impact	Attrition	Friction	Abrasion
Hourly throughput (kg h ⁻¹)	8–15	85	450	1600	100–600	100–600	100

Sources – Shelling methods: Ahouansou et al. (2002); Dehulling methods: François (1988).

using one of the three dehulling methods such as attrition disc mill type Amuda[®], and motorized dehullers Engelberg and Mini-PRL. Characteristics of the dehullers are given in Table I.

To facilitate removal of pericarp and embryo in the case of the dehuller Engelberg, the grains were humidified to attain moisture content between 18 and 22%. As for the attrition disc mill, the grains were thoroughly washed; whereas they remained dry (moisture content less than 14%) for the dehuller Mini-PRL. The grains were dehulled once for 4–6 min. Fumonisin content was measured as described above just before and after dehulling.

Statistical analyses

SPSS programme for Window version 10.0 (SPSS Inc., Chicago, Illinois) was used to test the statistical significance of differences between treatments with one-way analysis of variance (ANOVA). Tukey HSD test was used to test differences between means of percentage damage caused on grain by each shelling method, means of *Fusarium* populations and mean fumonisin levels in maize. Pearson correlation test was used to evaluate relationships among percentage damage caused by the shelling methods, *Fusarium* incidence and fumonisin level.

Results and discussion

All the mechanical shelling methods which were tested caused damage to maize grains, with the percentage of damage by the IITA[®] sheller significantly higher than that of all the other methods ($p < 0.01$) (see Table II). This provides additional evidence that shelling methods can inflict damage on maize grains, some of them being able to be very damaging (Dharmaputra et al. 1994). It is likely that friction between grains and the cylinder of the sheller is the cause of important damage on grains. In this study, both the handle-operated sheller and the motorized Renson[®] sheller function similarly to the traditional method of shelling, which consists of rubbing cobs one against each other. Separation of

grains then occurs by friction. In contrast, the IITA[®] sheller functions similarly to the traditional method of beating cobs with a stick, after they have been placed in a bag. The cobs are beaten with beaters inside the shelling chamber. Grains are released from the cob's core due to impact between cobs and beaters, cobs and the inner surface of the shelling chamber, and the cobs against themselves while in their disordered movement inside the chamber. This may explain the high number of damaged grains found using this method.

There are other factors that may increase the risk of grain damage such as grain moisture content, insect damage and speed of sheller rotary cylinder. Percentage of grain damage increases if the grains are shelled at moisture levels higher than 18% (Dharmaputra et al. 1996). Grains damaged by insects and those having apparent cracks probably due to stress during the grain-filling period or excessive drying rates were also found to be more easily damaged or broken by shellers (Ahouansou et al. 2002). Higher speeds of the rotary cylinder inside the sheller ($> 500 \text{ r min}^{-1}$) are more likely to cause increased impact between the cobs and the shelling chamber, and between the cobs themselves, leading to more damage on grains (Ahouansou et al. 2002).

The number of *Fusarium* colonies was found to be higher in maize shelled with the mechanical shellers (see Table III), the highest number being in maize shelled using the IITA[®] sheller ($p < 0.05$). The number of colonies in maize shelled using the handle-operated sheller and the motorized Renson[®] sheller was not significantly different from that found in maize shelled by hand ($p > 0.05$). *Fusarium* population was positively and significantly correlated with the percentage of damage ($r = +0.6$; $p < 0.01$). This confirms that damage caused on grain due to mechanical shelling may serve as entry points for *Fusarium* fungi (Dharmaputra et al. 1994). Douglas and Boyle (1996) reported that multistage postharvest handling of grain, including shelling, increases grain damage and cracking, providing an opportunity for fungi to develop and penetrate the grain. GASGA (1997) has, therefore, stressed that grain damage should be minimized in order to reduce fungal infection.

Fumonisin levels were higher in maize shelled using the mechanical shellers, the highest being determined in maize shelled using the IITA[®] sheller ($p < 0.01$) (see Table IV). The levels detected in maize shelled using the handle-operated sheller and motorized Renson[®] sheller were not significantly different from that detected in maize shelled by hand ($p > 0.05$). Fumonisin levels positively and significantly were correlated with both the percentage of damage caused by the shelling methods ($r = +0.6$;

Table II. Mean percentage of damage caused to maize grains by different shelling methods.

Shelling methods	n*	Mean percentage of damage (%)**
Shelling by hand	3	0 a
Handle-operated sheller	3	1.0 ± 0.2 b
Motorized sheller type Renson [®]	3	0.9 ± 0.7 b
Motorized sheller type IITA [®]	3	3.5 ± 0.8 c

*Number of maize samples submitted to each shelling method during the experiment; **Means in column followed by the same letter indicate no significant difference by Tukey's test ($p < 0.05$).

Table III. Mean *Fusarium* population in maize samples during 3-month storage period.

Shelling methods	n*	Population of <i>Fusarium</i> (cfu g ⁻¹)**			
		0 month after stocking	1 month after stocking	3 months after stocking	Mean over 3 months of storage
Shelling by hand	3	1766.7 ± 208.2 a	1700.0 ± 264.6 a	1466.4 ± 461.9 a	1644.4 ± 316.7 a
Handle-operated sheller	3	2066.7 ± 929.2 a	1766.7 ± 115.5 a	1533.3 ± 57.7 ab	1788.9 ± 523.1 a
Motorized sheller type Renson®	3	1933.3 ± 776.8 a	2000.0 ± 556.8 a	1700.0 ± 435.9 ab	1877.9 ± 542.6 a
Motorized sheller type IITA®	3	2033.3 ± 208.2 a	3100.0 ± 200.0 b	2466.6 ± 321.5 b	2533.3 ± 512.4 b

*Number of maize samples submitted to each shelling method during the experiment; **Means in columns followed by the same letter indicate no significant difference by Tukey's test ($p < 0.05$).

Table IV. Mean total fumonisin level in maize samples during 3-month of storage period.

Shelling methods	n*	Fumonisin level in maize (mg kg ⁻¹)**			
		0 month after stocking	1 month after stocking	3 months after stocking	Mean over 3 months of storage
Shelling by hand	3	1.6 ± 0.1 a	0.3 ± 0.1 a	nd a**	0.7 ± 0.7 a
Handle-operated sheller	3	1.5 ± 0.2 a	1.3 ± 0.2 b	0.5 ± 0.1 a	1.1 ± 0.5 a
Motorized sheller type Renson®	3	1.5 ± 0.1 a	0.9 ± 0.1 c	0.5 ± 0.2 a	1.0 ± 0.5 a
Motorized sheller type IITA®	3	1.6 ± 0.1 a	3.2 ± 0.1 d	1.7 ± 0.2 b	2.2 ± 0.8 b

*Number of maize samples submitted to each shelling method during the experiment; **nd = not detected = level < 0.25 mg kg⁻¹ of fumonisins using VICAM method; ***Means in columns followed by the same letter indicate no significant difference by Tukey's test ($p < 0.05$).

$p < 0.01$) and the number of *Fusarium* colonies in maize ($r = +0.7$; $p < 0.01$). This finding is in agreement with Nelson et al. (1993) who showed production of mycotoxin to be significantly affected by factors such as grain damage. Fumonisin levels in maize shelled using the IITA® sheller increased during the first month of storage (see Table IV), presumably due to the fact that *Fusarium* infection was still very active. This result is consistent with the fact that fumonisin levels were positively and significantly correlated with the number of *Fusarium* colonies found on the samples.

With respect to dehulling methods, fumonisin levels significantly decreased in maize after dehulling ($p < 0.01$) (see Figure 1). This decrease was not, however, significantly different from one dehulling method to another ($p > 0.05$). The dehuller Mini-PRL induced a mean reduction of 62% of fumonisin level (from 0.65 mg/kg before dehulling to 0.25 mg/kg after dehulling), the dehuller Engelberg, 65% (0.71–0.25 mg/kg) and the attrition disc mill, 57% (0.68–0.29 mg/kg). Fumonisin is likely to be more concentrated in the outer parts (pericarp and embryo) of the maize grain so that removal of these parts would result in a reduction of the toxin level in maize (Sydenham et al. 1995, Katta et al. 1997). Trenholm et al. (1991) found dehulling to result in a 40–100% reduction in the *Fusarium* toxins deoxynivalenol and zearalenone in contaminated barley, wheat and rye.

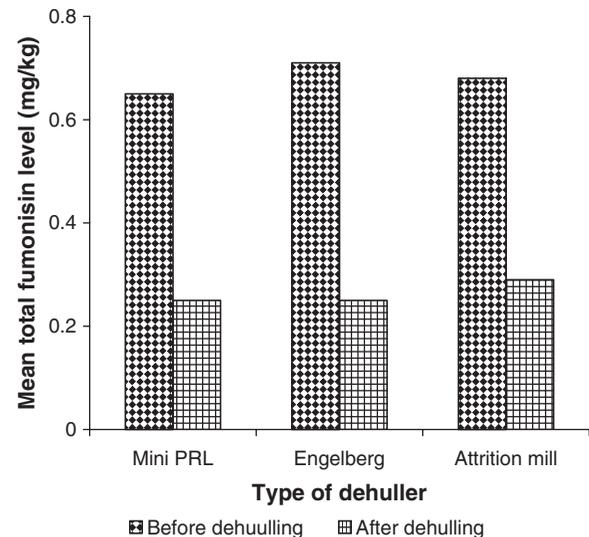


Figure 1. Mean fumonisin level in maize before and after dehulling using different dehulling methods.

No significant differences in fumonisin levels were observed for the tested dehulling methods. However, both dehullers Mini-PRL and Engelberg appear to have been more efficient than the attrition disc mill in grain dehulling, inducing a numerically better reduction of fumonisin levels. The mill is commonly used in West Africa for maize milling, but it does not seem to be adapted for maize dehulling when

compared to the Mini-PRL. The attrition disc mill possesses two discs, one fixed and the other mobile, and during the dehulling operation, the gap between these discs needs to be regularly adjusted to avoid grain breakage, which complicates its use (François 1988).

Conclusions

This study has clearly shown that shelling and dehulling are important steps in the processing of maize with respect to mycotoxin contamination. In particular, mechanical dehulling significantly reduced fumonisin levels and can be recommended as a decontamination method. Much more attention should be given to this processing operation that should be widely developed mainly in the African countries where it is still uncommon. Whereas automated shelling machines are being increasingly promoted in Africa to reduce the drudgery of food processing to farmers, mainly to women, introducing appropriate machines that are less damaging should be a great challenge. Moreover, it is very important to stress that efforts should be made by the farmers to always meticulously remove damaged grains from maize bulk to reduce fungal infection and mycotoxin level.

Acknowledgments

We gratefully acknowledge the financial support of the Danish International Development Assistance (DANIDA) and the International Institute of Tropical Agriculture (IITA) for implementing the studies on which this manuscript is based. We are especially grateful to Mariam Bouraïma, Benoit Gnonlonfin and Claudine Adimou for their technical assistance.

References

- Ahouansou R, Fandohan P, Adegbola P, Singbo A. 2002. Etude technique et économique des égreneuses à maïs au Bénin. Programme Technologie Agricole Alimentaire, Programme Analyse de Politique Agricole. Available from: Institut National des Recherches Agricoles du Bénin.
- Bolger M, Coker RD, DiNovi M, Gaylor D, Gelderblom W, Olsen M, Paster N, Riley RT, Shephard G, Speijers GJA. 2001. Fumonisin. In: Safety evaluation of certain mycotoxins in food, WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva. pp 103–279.
- Chu FS, Li GY. 1994. Simultaneous occurrence of fumonisin B1 and other mycotoxins in mouldy corn collected from the People's Republic of China in region with high incidence of oesophageal cancer. *Applied and Environmental Microbiology* 60:847–852.
- Dharmaputra OS, Purwadaria HK, Susilo H, Ambarwati S. 1994. The effects of drying and shelling on *Fusarium* spp. Infection and *Fusarium* toxins production in maize. Available: http://library.biotop.org/administrative_report.htm, via the INTERNET. Accessed 14 July 2004.
- Dharmaputra OS, Retnowati I, Purwadaria HK, Sidik M. 1996. Surveys on postharvest handling, *Aspergillus flavus* infection, and aflatoxin contamination of maize collected from farmers and traders. In: Highley E, Johnson GI, editors. Mycotoxin contamination in grains. Papers presented at the 17th ASEAN Technical Seminar on Grain Postharvest Technology, Lumut, Malaysia, 25–27 July 1995, Australian Centre for International Agricultural Research, Canberra. pp 38–53.
- Douglas PL, Boyle R. 1996. Effect of drying control on mycotoxin production. In: Highley E, Johnson GI, editors. Mycotoxin contamination in grains. Papers presented at the 17th ASEAN Technical Seminar on Grain Postharvest Technology, Lumut, Malaysia, 25–27 July 1995, Australian Centre for International Agricultural Research, Canberra. pp 27–33.
- Fandohan P. 2004. *Fusarium* infection and mycotoxin contamination in preharvest and stored maize in Benin, West Africa. PhD Thesis. University of Pretoria, South Africa. 196 p.
- FAO. 1992. L'après-récolte des grains: Organisation et techniques. Bulletin des Services Agricoles de la FAO no. 93. Organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO), Rome.
- François M. 1988. Du grain à la farine: le décorticage et la mouture des céréales en Afrique de l'Ouest. Collection le Point sur les Technologies. Ministère de la Coopération et du Développement Français (CF), Centre Technique de Coopération Agricole et Rurale (CTA), Groupe de Recherche et d'Echanges Technologiques (GRET), France.
- GASGA. 1997. Mycotoxins in grain. Technical Leaflet no. 3. Group for Assistance on Systems Relating to Grain After Harvest (GASGA), Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, The Netherlands.
- Harrison LR, Colvin BM, Greene JT, Newman LE, Cole JR. 1990. Pulmonary oedema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigations* 2:217–221.
- Houssou P. 2000. Storage and packaging studies on degermed maize flour. MPhil Thesis, Department of Nutrition and Food Science. Available from: University of Ghana, Legon, Ghana.
- Katta SK, Cagampang AE, Jackson LS, Bullerman LB. 1997. Distribution of *Fusarium* moulds and fumonisins in dry-milled corn fractions. *Cereal Chemistry* 74:858–863.
- Kellerman TS, Marasas WFO, Thiel PG, Gelderblom WCA, Cawood ME, Coetzer JAW. 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B₁. *Onderstepoort Journal of Veterinary Research* 57:269–275.
- Kozakiewicz Z. 1996. Occurrence and significance of storage fungi and associated mycotoxins in rice and cereal grains. In: Highley E, Johnson GI, editors. Mycotoxin contamination in grains. Papers presented at the 17th ASEAN Technical Seminar on Grain Postharvest Technology, Lumut, Malaysia, 25–27 July 1995, Australian Centre for International Agricultural Research, Canberra. pp 18–26.
- Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species. An illustrated manual for identification. The Pennsylvania State University Press, University Park and London.
- Nelson PE, Desjardins AE, Plattner RD. 1993. Fumonisin, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. *Annual Review of Phytopathology* 31:233–252.
- Pantenius CU. 1988. Etat des pertes dans les systèmes de stockage du maïs au niveau des petits paysans de la région maritime du Togo. GTZ. Germany.

- Rheeder JP, Marasas WFO, Thiel PG, Sydenham EW, Shephard GS, Van Schalkwyk DJ. 1992. Fusarium moniliforme and fumonisins in corn in relation to human oesophageal cancer in Transkei. *Phytopathology* 82:353–357.
- Schulthess F, Cardwell KF, Gounou S. 2002. The effect of endophytic *Fusarium verticillioides* on infestation of two maize varieties by lepidopterous stemborers and coleopteran grain feeders. *Phytopathology* 92:120–128.
- Sydenham EW, Stockenstrom S, Thiel PG, Shephard GS, Koch KR, WFO Marasas. 1995. Potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. *Journal of Agricultural and Food Chemistry* 43:1198–1201.
- Trenholm HL, Charmley LL, Prelusky DB, Warner RM. 1991. Two physical methods for the decontamination of four cereals contaminated with deoxynivalenol and zearalenone. *Journal of Agricultural and Food Chemistry* 39:356–360.
- Ueno Y, Iijima K, Wang SD, Suguira Y, Sekijima M, Tanaka T, Chen C, Yu SZ. 1997. Fumonisins as a possible contributing risk factor for primary liver cancer: A 3 year study of corn harvested in Haimen, China by HPLC and ELISA. *Food Chemistry and Toxicology* 35:1143–1150.
- VICAM. 1998. FumoniTestTM Instruction Manual VICAM, Watertown. p 39.