CHAPTER FIVE

IMPACT OF MECHANICAL SHELLING AND DEHULLING ON *FUSARIUM* INFECTION AND FUMONISIN CONTAMINATION OF MAIZE

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ABSTRACT

Mechanical shelling and dehulling methods were tested to evaluate their impact on *Fusarium* infection and fumonisin contamination in maize. The mechanical shelling methods tested were found to damage the grains. The motorised sheller type IITA caused the highest level (up to 3.5 %) of damage. This could be due to the operation mode of that machine. *Fusarium* populations were higher on damaged grains. The highest number of colonies was recorded from grains damaged by the IITA sheller (2533.3 cfu g^{-1}). Total fumonisin levels were also higher in damaged grains, the highest being in maize shelled by 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 ($r = + 0.6$, $p < 0.01$), and also with the number of *Fusarium* colonies from maize ($r = + 0.7$, $p < 0.01$). In contrast to the other shelling methods, an increase of the fumonisin level was observed during the first month of storage in maize shelled with the IITA sheller. On the other hand, the mechanical dehulling methods reduced fumonisin levels in maize. The use of dehullers resulted in a reduction of 64 – 68 % for Mini-PRL, 62 – 67 % for Engelberg, and 56 – 62 % for the attrition disc mill. This study has clearly shown the effects of shelling and dehulling methods on fungal infection and mycotoxin contamination of maize. It is important for farmers to choose appropriate shelling methods to reduce mycotoxin contamination. Also, dehulling, which is an important step in the processing of maize in Africa should be widely promoted for the reduction of mycotoxins in maize. This is a major challenge for all agricultural institutions in Africa.

Key words: Maize, mechanical shelling, dehulling, *Fusarium*, fumonisins, Benin

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. It is often accompanied by degerming (removal of the embryo).

Shelling and dehulling are generally executed by women, and are very labour intensive and time consuming (Diop *et al.* 1997). 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, Diop *et al.* 1997). 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, Diop *et al.* 1997).

Different types of mechanical equipment have been introduced in rural and urban areas of Africa to make shelling and dehulling of maize easier, faster and more efficient. Observations in the field indicated that some of this equipment cause damage on grains promoting serious fungal infection (Fandohan *et al.* 2002, unpublished data). Up to now, little attention has been given to the possible effects that these machines may have, not only on fungal infection but also mycotoxin contamination of maize. Kozakiewicz (1996) stressed that postharvest mechanisation in general, if not used correctly, can damage the processed products and may facilitate fungal infection. Dharmaputra *et al.* (1996) reporting results from national surveys in Indonesia, found strong evidence that maize shelling could cause mechanical damage, allowing fungi to infect grains. Some of these fungi can produce toxic substances called mycotoxins. Examples are toxigenic *Fusarium* spp. producing fumonisins in maize (Munkvold and Desjardins 1997).

Fumonisins are recently identified mycotoxins (Gelderblom *et al.* 1988) mainly produced by *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) Nirenberg. Since their discovery, fumonisins have attracted increasing attention because of their adverse effects on animal and human health and their negative economic impact (Bolger *et al.* 2001). They have 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 and Li 1994) and liver cancer (Ueno *et al.* 1997).

The present study was undertaken to elucidate the impact of automated shelling and dehulling methods currently promoted in West Africa on *Fusarium* infection and fumonisin contamination of maize. The main objective was to draw more attention to the effects that machines may have on mycotoxin contamination of maize, in order to alert agricultural institutions and farmers to take these factors into account when choosing equipment.

MATERIAL AND METHODS

Maize cultivar used

The maize used in this study was the 90-day cultivar DMR-ESR-W, which is an improved IITA (International Institute of Tropical Agriculture) white and hard grain variety. Maize cobs were obtained from the Benin Station of IITA, Abomey-Calavi, situated in the Forest Mosaic Savannah of Benin. 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 were immediately dehusked after harvest 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 different methods with one shelling method for each lot (Fig 1, 2, 3 & 4). These methods included shelling by hand, shelling using a handle-operated sheller, and shelling using two motorised shellers type Renson, France and type IITA, Nigeria. Characteristics of the shellers are described in Table 1. Grains (10 kg) from each lot were stored in weaved polyethylene bags at room temperature (28 - 30°C) for three months. There were three bags per treatment (shelling method) as each treatment was replicated three times. Prior to storage, grains in each bag were dusted with the binary insecticide Sofagrain® (0.05 % deltamethrin and 1.5 % pirimiphos-methyl) to reduce insect damage.

A 500 g-sample was taken from each bag (Fig 5) at the beginning of the trial, and after 1 and 3 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, whereas damage caused by insects was assessed before shelling (Pantenius 1988). In order to reduce the eventual influence of grain moisture content, damage on grain by insects and sheller speed, the cobs were sun-dried prior to shelling to bring the grain moisture content to a level less than 18 %. 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^{-1}.

Fusarium species were enumerated using dilution plating at the beginning of the trial, and also at 1 and 3 months after stocking. A 10 g sub-sample of maize grains was taken from each bag, 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 exposed to a 12:12-hour light/dark regime for 5 days. *Fusarium* colonies were isolated and transferred onto carnation leaf agar and incubated for 7 days at 25 °C exposed to a 12:12-hour 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 as described in Chapter 2 at the beginning of the trial, and after 1 and 3 months of storage using the VICAM method (VICAM 1998).

Impact of different dehulling methods on Fusarium- and fumonisin contamination

Grains from the bags of maize initially shelled with the two motorised shellers were thoroughly mixed after 3 months of storage and divided into three lots of approximately 7 kg each (Fig 5). Three replicates of 2 kg of maize were sampled from each lot and dehulled using one of the following three different dehulling methods, i.e. attrition disc mill type Amuda, and motorised dehullers Engelberg and Mini-PRL (Fig 6, 7 & 8). Characteristics of the dehullers are given in Table 1. To facilitate removal of pericarp and embryo, the grains were humidified to attain moisture content between 18 and 22 % in the case of the dehuller Engelberg. Grains were thoroughly washed for the attrition disc mill, but 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 program 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 performed to test differences between means of percentage damage caused on grain by each shelling method, means of *Fusarium* populations and mean levels of fumonisin in maize samples. Pearson correlation test was used to evaluate relationships among percentage damage caused by the shelling methods, *Fusarium* incidence and fumonisin level.

RESULTS

Impact of different shelling methods on Fusarium- and fumonisin contamination

Mechanical shelling methods caused damage to grain (Table 2). The percentage of damage caused by the IITA sheller was significantly higher than that of all the other methods ($p < 0.01$). The handle-operated sheller and motorised Renson sheller caused significantly higher damage than hand shelling ($p < 0.01$), but there was no significant difference between the percentages of damage caused by each other ($p > 0.05$).

Mycological analyses showed that *F. verticillioides* and *F. proliferatum* were the *Fusarium* spp. found in the maize samples during the study. The former was encountered in all the samples whereas the latter was found only in the samples shelled by hand. The number of *Fusarium* colonies was higher in maize shelled with the mechanical shellers (Table 3). The number of colonies from maize shelled using the IITA sheller was significantly higher than in maize shelled using the other shelling methods ($p < 0.05$). *Fusarium* population in maize shelled using the two other mechanical shelling methods (use of handle-operated sheller and motorised Renson sheller) was not significantly different from that found in maize shelled by hand ($p > 0.05$). The number of *Fusarium* colonies found in maize was positively and significantly correlated with the percentage of damage ($r = +0.6$, $p < 0.01$). *Fusarium* populations in maize changed throughout the 3-month storage period (Table 3). This change was, however, significant ($p < 0.01$) only in maize shelled using the IITA sheller, *Fusarium* populations increasing from 2033.3 cfu g^{-1} at the beginning to 3100.0 cfu g^{-1} after 1 month, before decreasing at 3 months of storage (Table 3).

Mean fumonisin levels were found to be higher in maize shelled using the mechanical shellers (Table 4). The highest mean level was detected in maize shelled using the IITA sheller, and that was significantly different from the level found in maize shelled using other shelling methods ($p < 0.01$). Fumonisin levels detected in maize shelled using the handle-operated sheller and motorised Renson sheller were not significantly different from that detected in maize shelled by hand ($p > 0.05$). There was a positive and significant correlation between the fumonisin levels in maize and the percentage of damage caused by the shelling methods ($r = + 0.6$, $p < 0.01$). The fumonisin levels were also positively and significantly correlated with the number of *Fusarium* colonies from the maize samples ($r = + 0.7$, $p < 0.01$).

Changes were also observed in the fumonisin level in maize throughout the storage period, and in contrast to *Fusarium* populations, these changes were significant in all cases ($p < 0.01$) (Table 4). The fumonisin level increased in maize shelled using the IITA sheller from 1.6 mg kg^{-1} at the beginning to 3.2 mg kg^{-1} after 1 month before decreasing to 1.7 mg kg^{-1} at 3 months of storage, whereas the level markedly decreased in the maize shelled using the other shelling methods (67 – 90 % of reduction of fumonisin level) (Table 4).

Impact of different dehulling methods on Fusarium- and fumonisin contamination

Fumonisin levels significantly decreased in maize after dehulling ($p < 0.01$) (Fig. 9). This decrease was not, however, significantly different from one dehulling method to another ($p > 0.05$). The dehuller Mini-PRL induced a reduction of 64 – 68 %, the dehuller Engelberg, 62 – 67 % and the attrition disc mill, reduced levels by 56 – 62 %.

DISCUSSION

Results of this study provide firm evidence that methods of shelling can inflict damage on maize grains. Some mechanical shelling methods can be very damaging. Dharmaputra *et al.* (1994) noticed a higher percentage of damaged grains in maize shelled mechanically (5.7 %) than that in maize shelled using a nailed wood used as a sheller (2.9 %). In a previous study, Suprayitno (1980) has suggested that friction between grains and the cylinder of the sheller could cause a high number of damaged grains after mechanical shelling.

Both the handle-operated sheller and the motorised Renson sheller tested in this study 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 some other factors that may increase the risk of grain damage. Percentage of grain damage increases if the grains are shelled at moisture levels higher than 18 % (Dharmaputra *et al.* 1994, Dharmaputra *et al.* 1996). Grains damaged by insects and those having apparent cracks probably due to stress during grain-filling period or excessive drying rates were found to be more easily damaged or broken by shellers (Ahouansou *et al.* 2002). Higher speeds of the rotary cylinder inside the sheller (more than 500 r min^{-1}) is 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).

Fusarium infections were more common on damaged grains. This indicates that damage caused on grain due to mechanical shelling may serve as entry points for *Fusarium* fungi. Dharmaputra *et al.* (1994) found *Fusarium* populations on maize shelled with a mechanical sheller to be higher (7129 cfu g^{-1}), but not significantly different to those on maize shelled with a nailed wooden instrument (5044 cfu g^{-1}). Similarly, 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) have, therefore, stressed that grain damage should be minimised in order to reduce fungal infection.

Levels of fumonisin were higher in maize shelled using mechanical shellers, with the highest level being detected in maize shelled using the IITA sheller. Fumonisin level was also found to significantly correlate positively with the percentage of damaged grains. 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. An increase of fumonisin levels was detected in samples of maize shelled using the IITA sheller after the first month of storage. This could presumably be due to the fact that at that time, *Fusarium* infection was still very active in maize of these samples. The population of *Fusarium* was also found to be higher in these samples, and grain moisture content, still around 18 % in the first month of storage (Table 5), might allow for development of fungi and mycotoxin production. 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.

Mechanical dehulling significantly reduced fumonisin levels in maize. This confirms evidence that fumonisin is likely to be more concentrated in the outer parts (pericarp and embryo) of the maize grain. Removal of these parts can markedly reduce the fumonisin level in maize (Sydenham *et al.* 1994, Sydenham *et al.* 1995, Katta *et al.* 1997, FDA 2001, Voss *et al.* 2001). 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. In a more recent study in Benin, a reduction of up to 63 % of fumonisin was observed due to dehulling of maize during the preparation of maize-based foods (Chapter 4).

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 but not statistically significantly 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 mill possesses two discs, one fixed and the other mobile. During the dehulling operation, the gap between these discs needs to be regularly adjusted to avoid grain breakage (François 1988).

This study has clearly shown that shelling and dehulling are important steps in the processing of maize in Africa with respect to the reduction of mycotoxin levels. In particular mechanical dehulling significantly reduced fumonisin levels and can be recommended as a decontamination method in African countries where maize is a staple diet. Much more attention should be given to this processing operation that should be widely developed in the African countries where it is still uncommon. Moreover, 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 for African research institutions. It is also 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. The implementation of appropriate sorting, mechanical shelling and dehulling methods is therefore a major challenge for all agricultural institutions in Africa.

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Table 1: Characteristics and use conditions of the different tested shelling and dehulling methods

Characteristics	**Shelling methods**				**Dehulling methods**		
	Shelling by hand	**Handle-operated sheller**	**Motorised sheller type Renson**	**Motorised sheller type IITA**	**Attrition disc mill**	**Engelberg**	**Mini-PRL**
Manufacturer	-	Renson (France)	Renson (France)	IITA (Nigeria)	Amuda (India)	Rajan (India)	Pere et Frere (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)	8 – 15	85	450	1600	100 - 600	100 - 600	100

Sources:

- Shelling methods : Ahouansou et al. (2002)
- Dehulling methods : François (1988), Diop et al. (1997)

Table 2: 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
Motorised sheller type Renson	3	0.9 ± 0.7 b
Motorised sheller type IITA	3.	3.5 ± 0.8 c

n = number of maize samples on which damage caused by the shelling methods was assessed

Means within a column followed by the same letter are not significantly different ($p > 0.05$)

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

Shelling methods	n	Population of *Fusarium* (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	1466.7 ± 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 a	1788.9 ± 523.1 a
Motorised sheller type Renson	3	1933.3 ± 776.8 a	2000.0 ± 556.8 a	1700.0 ± 435.9 a	1877.9 ± 542.6 a
Motorised sheller type IITA	3	2033.3 ± 208.2 a	3100.0 ± 200.0 b	2466.7 ± 321.5 a	2533.3 ± 512.4 b

n = number of maize samples on which *Fusarium* population was assessed

Means within a row followed by the same letter are not significantly different ($p > 0.05$) from each other.

Table 4: 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
Motorised sheller type Renson	3	1.5 ± 0.1 a	0.9 ± 0.1 c	0.5 ± 0.2 a	1.0 ± 0.5 a
Motorised sheller type IITA	3	1.6 ± 0.1 a	3.2 ± 0.1d	1.7 ± 0.2 b	2.2 ± 0.8 b

Means in columns followed by the same letter are not significantly different ($p > 0.05$) from each other.

nd = not detected = level < 0.25 mg kg^{-1} of fumonisins.

Table 5: Mean moisture content of grains during 3-month of storage period

Shelling methods	**n**	**Grain moisture content (%)**		
		0 month after stocking	**1 month after stocking**	**3 months after stocking**
Shelling by hand	3	20.4 ± 0.6	17.4 ± 0.8	14.0 ± 0.2
Handle-operated sheller	3	20.4 ± 0.5	17.2 ± 0.1	13.6 ± 0.1
Motorised sheller type Renson	3	20.7 ± 0.5	17.2 ± 0.4	14.0 ± 0.3
Motorised sheller type IITA	3	20.4 ± 0.5	17.5 ± 0.4	13.9 ± 0.9

Figure 1: Manual shelling operation

Figure 2: A handle-operated sheller (Renson)

Figure 3: A motorised sheller (Renson)

Figure 4: A motorised sheller (IITA)

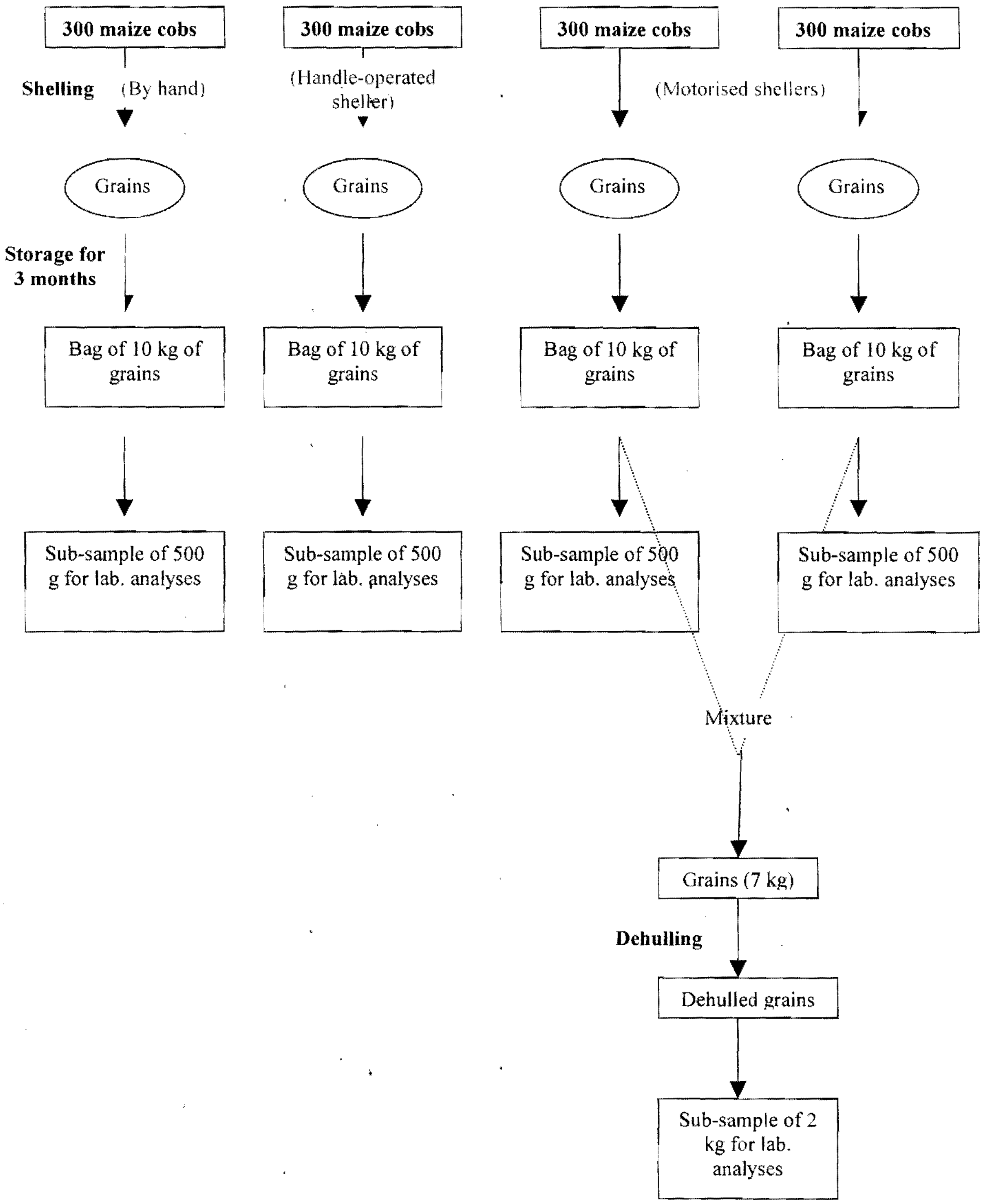

Figure 5: Diagram summarising the methods used during this study

Figure 6: Engelberg motorised dehuller

Figure 7: Mimi-PRL dehuller

Figure 8: An attrition disc mill (Amuda)

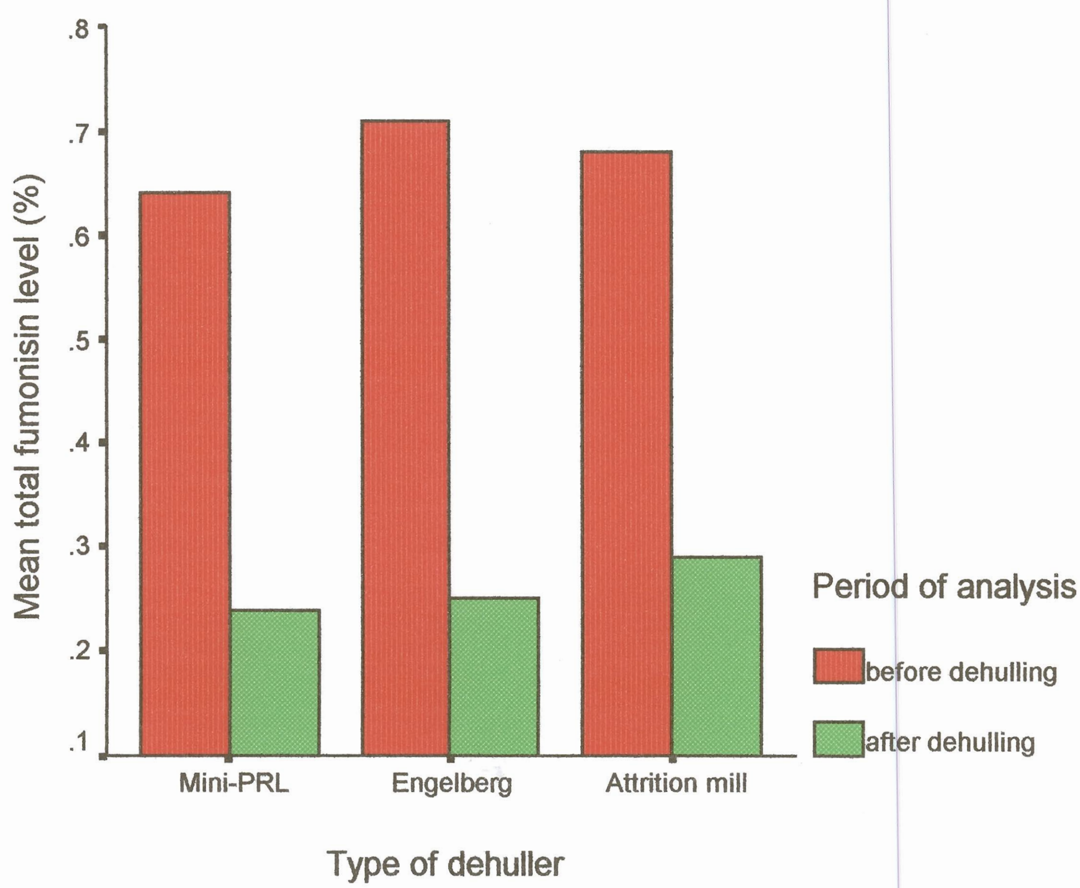

Figure 9: Mean fumonisin level in maize before and after dehulling using different types of dehulling methods