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Full Length Research paper

Natural occurrence of AFB1 in maize and effect of traditional maize flour production on AFB1 reduction in Malawi

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Aflatoxin B_1 (AFB_1) is one of the most harmful mycotoxins to humans, animals, and crops that result in illnesses and economic losses. Maize, the most important staple food in most southern African countries, is susceptible to these fungi infections throughout its growth, harvest, transport and storage. In this study, we report on the incidence of AFB_1 (determined by indirect competitive ELISA) contamination in maize from Malawi and the effect of traditional maize- flour production procedures on the final AFB_1 levels. AFB_1 was detected in 45.3% of the maize samples with 12.3% of them exceeding 5 µg/kg (FAO median AFB_1 MTL). The traditional flour production procedures reduced AFB_1 significantly in the order: soaking of dehulled maize (72.4±5.4, 75.4±3.5 and 80.9±5.3% for 24, 48 and 72 h soaking periods, respectively) > dehulling of maize (mean 29.3±5.4%) > sun drying (11.7% max). Sun drying followed pseudo-first order kinetics in AFB_1. A maximum AFB_1 reduction of 88.1 ± 3.1% was achieved using a sequence of dehulling, soaking for 72 h and sun drying the flour for 4.5 h.

Key words: Aflatoxin B_1, maize, Malawi.

INTRODUCTION

Maize (*Zea mays)* is the major staple food in the developing world, mostly in the Sub-Saharan Africa. In Malawi, maize crop is cultivated on more than 70% of the total arable land and contributes significantly to diets of more than 80% of the population, with per capita consumption of 182 kg per year (Pingali, 2001). However, maize is susceptible to contamination by aflatoxins, fungal metabolites that contaminate agricultural products and threaten food safety. They are produced by three species of *Aspergillus*, namely *A. flavus, A. parasiticus* and *A. nomius*. Aflatoxins have been shown in many studies to be hepatotoxic, teratogenic, mutagenic, genotoxic and hepatocarcinogenic, depending on the duration and level of exposure (Hendrickse, 1997; Peraica et al., 1999; Fung and Clark, 2004).

A series of studies on aflatoxin biomarkers have indicated that extensive exposure to aflatoxins can occur in humans of all ages in Africa (Coulter et al., 1984, 1986; Lamplugh et al., 1988; De Vries et al., 1989; Wild et al., 1991, 1992; Zarba et al., 1992; Jonsyn et al., 1995; Oyelami et al., 1996; El-Sayed et al., 2000; Gong et al., 2002, 2003, 2004). Further, there have been various reported outbreaks of human aflatoxicosis in Africa (Muraguri et al., 1981; Nangindu et al., 1982; Siboe and Muriuki, 1995; Lewis et al., 2005; Probst et al., 2007). In general, the prevention of aflatoxin contamination is not possible, hence salvage mechanisms that reduce the toxin content should be utilised to the maximum extent feasible. The effect of several industrial processing methods on the reduction of aflatoxins in corn has been reported (Scott, 1984; Rustom et al., 1993; Samarajeewa et al., 1990; Rustom, 1997). Overall, varying degrees of

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Table 1. Sections and number of participating households in the survey on incidence of aflatoxin in maize and flour. Refer to Figure 1 for locations.

Location in	Number of participating households	
***Mpingu* EPA**	**Maize**	**Milled maize (flour)**
Kalima	11	-
Sinumbi	12	14
Masiye	13	15
Kadzio	13	13
Kafisi	13	-
Likuni	14	-
Kakule	14	-
CARS residence	16	-
Katate	-	15
Kawanda	-	15
Kagwanipenya	-	16
Total	106	88

aflatoxin reduction are reported, most being directly related to the nature of the specific physical or chemical processing technique and commodity.

Traditional methods used in the preparation of the final consumed product have also been reported to reduce aflatoxin content to varying degrees, depending on the process (Sennapa and Nyagahungu, 1982; Njapau et al., 1998; Fandohan et al., 2005; Mutungi et al., 2008). In Southern Africa, the final consumed product from maize is mostly a thick porridge; popularly known as *ugali* (e.g. in Tanzania, Kenya), *nshima* (e.g. in Zambia), *sadza* (e.g. in Zimbabwe) or *nsima* (in Malawi).

In Malawi, little work has been done to assess status of aflatoxin contamination in foodstuffs and this has mainly focused on groundnuts, due to their importance in trade. However, contaminated maize has been blamed for aflatoxic hepatitis outbreaks in Kenya and India (Bulatao-Jayme et al., 1976). This is supported by studies conducted in Thailand, Philippines and USA that revealed that dietary intake of aflatoxins from groundnuts was lower than that from maize (Mehan et al., 1991) . A recent survey in Makueni District (Kenya) showed that 35.5% of maize had aflatoxin levels above the WHO MTL of 20 µg/kg with 20.1% presenting aflatoxin above 100 µg/kg (Mwihia et al., 2008). There is therefore need to assess the levels of aflatoxins in maize, as a staple food. The four major naturally occurring aflatoxins are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). AFB_1 is the most toxic in this series and has been classified as Group I human carcinogen by the International Agency for Research on Cancer (IARC) in 1987 (IARC, 1987). In this study, we therefore provide a first report on the incidence of AFB_1 contamination in maize from Malawi and the effect of traditional maize-flour production procedures on the final AFB_1 levels.

MATERIALS AND METHODS

Description of study site

Malawi is a small developing country situated between Zambia, Mozambique and Tanzania, in Southern Africa (Figure 1) . In common with greater part of South Central Africa, the country has distinct dry and wet seasons. The wet season lasts from November to May and the remainder of the year is dry, with temperatures increasing until the onset of the next rains. The study was conducted in *Mpingu* Agricultural Extension Planning Area (EPA), located about 20 km West of the Capital City, Lilongwe (Figure 1). The EPA is under Lilongwe Agricultural Development Division, one of the main maize production regions in Malawi. It has a mean annual temperature of 20°C, a mean annual relative humidity of 68% and receives a mean annual rainfall of 892 mm, 85% of which falls between November and March. The EPA has a total area of 26, 406 ha, 85.5% of which is arable land with about 14,800 farming families (Government of Malawi, 2008).

Sampling of flour and stored maize

Ninety households from seven of eighteen randomly selected sections of *Mpingu* EPA and sixteen households from *Chitedze* Agricultural Research Station (CARS) staff residence participated in the study (Table 1). From all households that participated in the survey, we collected a 5 kg shelled maize sample (farmers own produce) between 13 and 25 March, 2008. We also collected 51 whole-maize flour (locally known as *mgaiwa* flour) and 37 soaked dehulled maize flour (locally known as *woyera* flour) samples (200 g each) from 6 randomly selected maize mills located in 6 different sections of the EPA between 15 and 18 April, 2008 (Table 1). The samples were collected in khaki paper bags and stored at 0°C at CARS laboratory until analysis for aflatoxin.

Assessment of effect of traditional maize flour processes on AFB_1 levels

Artificial contamination of maize

The results of our survey on aflatoxin incidence in maize showed high heterogeneity and in most cases, the levels of AFB_1 were low to demonstrate statistically significant differences between treatments. We therefore, opted to contaminate Open Pollinated Variety (OPV623) maize acquired from a farmer from the study area in order to increase homogeneity and contamination level. 42.6 kg of maize (11.2% grain moisture content) was soaked in water for 6 h and then air-dried for 2 h, after draining the water. A one-litre suspension of aflatoxinogenic *aspergillus* species, prepared by washing heavily naturally contaminated de-husked maize, was spread over the maize and thoroughly mixed by hand. We then divided the inoculated maize into 10 equal portions, put the portions in moistened gunnysacks (Njapau et al., 1998) and spread them on racks of an incubator (Gallenkamp, Loughborough, UK) to a thickness of 4 - 5 cm. The maize was incubated at 29°C (Njapau et al., 1998) and on the seventh day, 200 mL of water was evenly sprayed over each maize portion to replenish lost moisture. Incubation was terminated after 14 days by air-drying the maize to 11.2% at 45°C. The 10 portions were recombined and thoroughly mixed.

In order to obtain practical levels of natural aflatoxin contamination, the incubated maize was divided into 10 portions by mass of 0.775, 1.549, 2.323, 3.098, 3.872, 4.647, 5.422, 6.196, 6.971 and 7.754 kg (corresponding to mass ratios of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, respectively). To each portion, we added healthy OPV623 maize to make a final mass of 22 kg. To

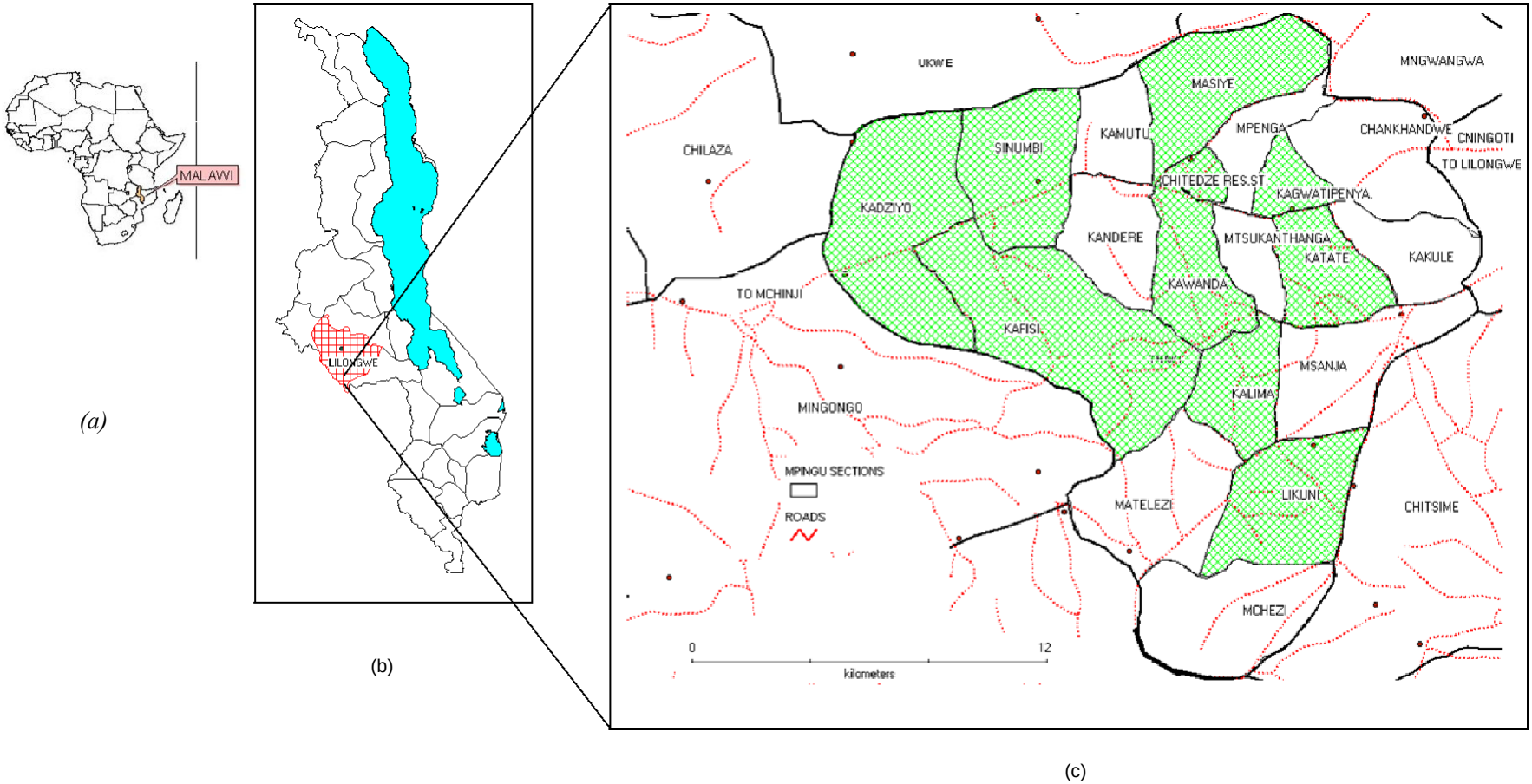

Figure 1. Map of Africa showing the location of Malawi *(a)*; map of Malawi showing location of *Mpingu* EPA *(b)* and sampled areas *(c)*.

maximize homogeneity the incubated and healthy maize portions were simultaneously poured slowly into a locally fabricated rotating mixer and mixed for 6 min.

Preparation of different types of flour

We established through interviews that households in the study area employed three methods of processing the maize into flour. These include (1) simply milling the maize to make whole meal flour (locally known as *mgaiwa* flour); (2) dehulling, soaking, drying and milling the grit into flour, followed by further drying (locally known as *woyera* flour) and (3) dehulling and thenknown as *gramil* flour). The three methods are summarized in Figure 2.

In order to simulate the flour production procedures of the village, we ground 4.54 kg from each of the ten portions (of the 22 kg-contaminated maize from §2.3.1 above) in a laboratory mill (Christy and Norris Ltd, Suffolk, UK) to pass sieve #20. From this, we prepared two different 200 g sub-samples. For the first sub-sample, we took the flour immediately after grinding and stored it at 0°C until aflatoxin assay (corresponding to sequence 1 in Table 2). The second one was taken from a 4½ h sun dried (mean temperature 27.6°C) flour that was evenly spread on 0.558 m^2 new sack surface and turned hourly before storing it at 0°C until aflatoxin analysis (sequence 2 in Table 2). 15 kg of each of the remainder from the ten contaminated maize (portions) was dehulled in a hammer mill located near CARS, using the expertise of three

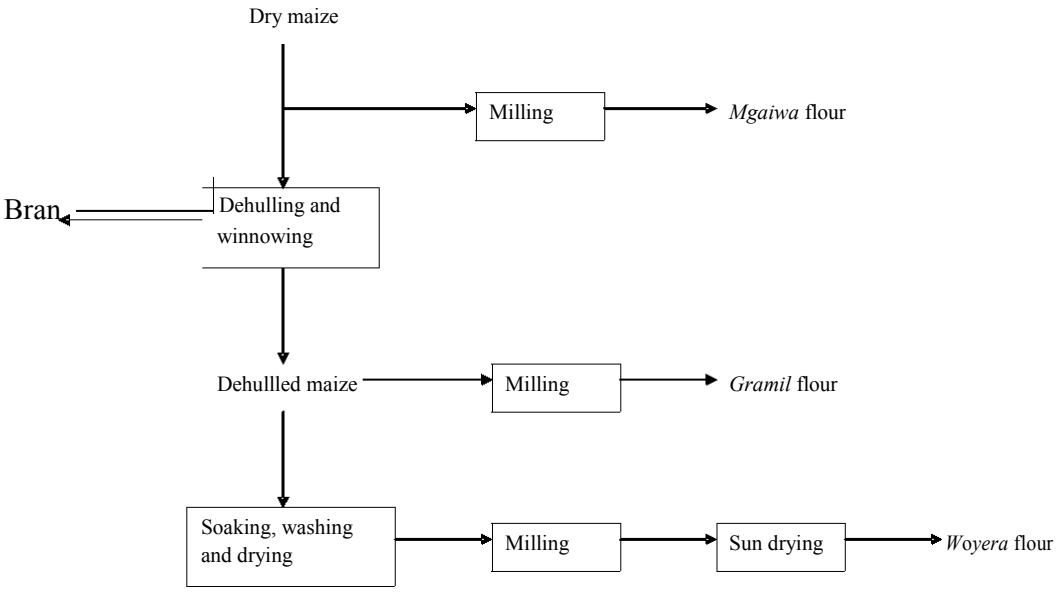

Figure 2. A flow sheet showing processes for production of the three flour types from dry maize, as is the practice in the study area.

Table 2. Sequence of procedures for flour production and mean cumulative percentage reduction in AFB_1 concentration during flour production. The AFB_1 levels and reduction percentages displayed are means of 10 replicates. Treatments with different letters in the *Remark* column have significantly ($p < 0.05$) different means according to Tukey's HSD test.

Sequence	Process	Final AFB_1 (µg/kg)	Induced percentage reduction in AFB_1 (%)	Remark
1	Milling	48.41 ± 26.75	0	Represents initial AFB_1 content in Maize
2	Milling sun drying (4½ h)	42.1 ± 22.4	11.7 ± 3.2	e
3	Dehulling milling	33.9± 18.9	29.3 ± 5.4	d
4	Dehulling milling sun drying (4½ h)	29.4 ± 15.3	37.3 ± 5.6	c
5	Dehulling soaking (24 h) milling	9.5 ± 6.1	80.9 ±4.4	b
6	Dehulling soaking (24 h) milling sun drying (4½ h)	8.6 ± 5.4	82.6 ±3.5	ab
7	Dehulling soaking (48 h) milling	8.6 ± 5.5	82.4 ±3.6	b
8	Dehulling soaking (72 h) milling	7.2 ± 5.3	86.5 ±3.5	a
9	Dehulling soaking (72 h) milling sun drying (4½ h)	6.4 ±5.3	88.1 ±3.1	a

hammer mill located near CARS, using the expertise of three women. Immediately before dehulling, the women added 2 L of water to the maize to enhance the dehulling process, in accordance with everyday village practice. The dehuller separated hulls from endosperm grits with 78 ± 4% efficiency. Further separation was achieved manually by use of a winnower. The two sets of bran (from dehuller and winnower) were combined, weighed and vigorously mixed by hand before taking a 2.5 kg sample. The bran sample was ground in a laboratory mill and 200 g of the resultant bran flour was stored at 0°C until aflatoxin assay.

We then divided 10 kg of the dehulled maize into 4 portions of 2.5 kg each using a sample divider (Burrows, Evanston, Illinois). Three of these samples were soaked in 2.5 L of groundwater in separate containers for 24, 48 and 72 h. The pH of soaking water was determined at the beginning of soaking and every 24 h for 72 h. At the end of the soaking period, the water was drained and the maize washed in a basin until wash water became clear. The maize was then sun dried for 1½ h. After this, we separately ground the three soaked portions and the fourth (non-soaked) portion in a laboratory mill, from which we drew two 200 g samples (one sun dried for 4.5 h and the other non-dried) for each portion. The samples were then stored at 0°C until aflatoxin assay (sequences 3 - 9 in Table 2).

DETERMINATION OF AFB_1 AND TOTAL AFLATOXINS

AFB_1 by indirect competitive ELISA Method

Indirect competitive ELISA Method (Waliyar et al., 2005) was used to determine AFB_1 in the maize and flour samples. Moisture content of test ground sample was determined by drying in an oven (ISO, 6540: 1980). A sample of flour, equivalent to 20 g dry matter, was

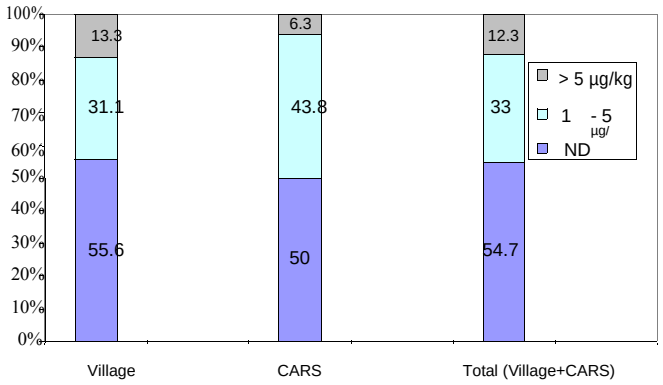

Figure 3. Incidence (%) of aflatoxin contamination in stored maize samples collected from farmers own produce in *Mpingu* EPA, Lilongwe, Malawi.
ND = not detected

mixed with 100 mL of a mixture of methanol and water (7:3 v/v) containing 0.5% KCl. The mixture was blended at high speed for 2 min and then transferred to a conical flask, shaken for 30 min at 300 rpm and then filtered through Whatman No. 41 filter paper. The filtrate was diluted in PBS-Tween (1:10). 150 µL of Aflatoxin B_1-Albumin bovine serum (AFB_1-BSA) conjugate in carbonate coating buffer (100 ng/mL) was dispensed into wells of a 96-well ELISA plate (Immuno 96 Microwell, Nunc-Maxisorp, Denmark) and incubated at 37°C for 1 h. The plate was washed in three changes of PBS-Tween allowing 3 min for each wash. Then 200 µL of 0.2% BSA in PBS-Tween was added to each well and incubated for 1 h. Blocked plate was washed with PBS-Tween only as described above. AFB_1 standard (Sigma AF–1, Sigma-Aldrich Co., St. Louis, MO, USA) was diluted at concentrations ranging from 25 µg/L to 0.0488 µg/L (using 1:2 diluted health maize extract).

Antiserum (50 µl) was added to each dilution of AFB_1 standard (100 µL) and test sample extract (100 µL) in triplicate and incubated at 37°C for 1 h. The plate was emptied and washed again as previously described. To each well 150 µL of goat anti-rabbit IgG labelled with alkaline phosphatase, in PBS-Tween containing 0.2% BSA (1:1000) was added and incubated for 1 h at 37°C. Plates were emptied and washed 3 times with PBS-Tween allowing 3 min for each wash. p-nitophenyl phosphate (PNPP) (150 µl) prepared in 10% diethanolamine buffer (pH 9.8) was added to each well and plate was kept in dark at 25°C for 1 h. Absorbance was measured at wavelength of 405 nm by means of an automatic ELISA reader (Multiskan EX, Thermo Electron Cooperation, France) . AFB_1 content was calculated using a reference curve of optical density (OD) values for AFB_1 standards plotted using graphical software Sigma Plot 8.0 (SPSS Inc., Chicago, IL) with AFB_1 concentration on the *x*-axis and optical density values on the *y*- axis. The method's limit of detection (LOD) was interpolated at 1.0 µg/kg. The amount of AFB_1 (ng/g) was calculated as follows

$$\mathrm{AFB_1}(ng/g) = \frac{A \times D \times E}{G} \quad (1)$$

Where *A* = AFB_1 concentration in sample extract (ng/mL); *D* = Dilution factor with buffer; *E* = Extraction solvent volume used (mL); and *G* = Dry matter weight equivalent (g).

Total aflatoxins by Vicam Fluorometer method

Further, 20 maize samples (from the maize survey) that were analyzed by indirect ELISA procedure for AFB_1 were also tested for total aflatoxins using the fluorometer aflatest immunoaffinity procedure (VICAM Inc., 1999) . A maize flour sample, equivalent to 50 g dry matter, was blended with 5 g of sodium chloride and 100 mL mixture of methanol and water (8:2 v/v) at high speed for 1 min using a blender. The mixture was filtered through fluted filter paper (Whatman 2V, Whatman, Middlex). The filtrate (15 mL) was diluted (1:4) with distilled water, re-filtered through glass microfibre filter paper (Whatman, Middlex, UK). The filtrate (10 mL) was passed through an aflatest inmunoaffinity column (VICAM, Watertown, MA, USA) at a rate of 1-2 drops/second. Distilled water (10 mL) was passed through the column at 1-2 drops/second and repeated once more until no bubbles come through the column. Toxin was eluted from the column with 1 mL HPLC grade methanol at the rate of 1-2 drops/second into a glass cuvette, mixed with freshly made 1 mL aflatest developer and its fluorescence measured in a pre-calibrated flourometer (VICAM V1 series 4, VICAM, Watertown, MA). LOD was interpolated at 1.0 µg/kg.

Statistical data analysis

The data from the survey of aflatoxin content in stored maize and flour samples were analysed using Statistical Package for Social Scientists (SPSS) for Windows version 12 (SPSS inc., Chicago, IL, USA). Differences between mean aflatoxin levels were tested by *t*-test. Descriptive statistics such as percentages and frequencies were used to summarize the data. Analysis of data from experiments on effect of different flour making procedures was performed using Stastisca 6.0 (StatSoft Inc.) software. The difference between treatment means was tested by ANOVA and Turkey's HSD test was used as a post ANOVA technique for pair-wise comparison of treatments.

RESULTS AND DISCUSSION

Occurrence of AFB_1 in stored maize and maize flour

AFB_1 was detected in 45.3% of the 106 stored maize samples analyzed, with 12.3 % of the maize exceeding the median AFB_1 Maximum Tolerable Limit (MTL) of 5 µg/kg (FAO, 2004) (Figure 3). The average AFB_1 in samples from village and CARS households was 1.76 ± 3.36 µg/kg and 1.48 ± 1.85 µg/kg respectively, without significant difference ($p > 0.05$) between them. The combined mean of the CARS and village household samples was 1.71 ± 3.17 µg/kg and the highest contaminated village and CARS sample registered AFB_1 content of 16.9 µg/kg and 5.3 µg/kg, respectively.

Out of the 20 samples analyzed for both AFB_1 and total aflatoxin, twelve samples (60%) had both detectable AFB_1 and total aflatoxin. We applied stepwise linear regression, using SPSS for Windows version 12 to the data for these samples to find a relationship between AFB_1 and total aflatoxin in stored maize from the study area. We obtained a strong and significant ($F = 68.76$, $p < 0.0001$, $r^2 = 0.78$) linear relationship (Figure 4) given by

$$\text{Total aflatoxin} = 1.3838 \times AFB_1 \quad (2)$$

Based on this relationship, we believe the toxin production to be dominated by *A. flavus* species. Malawi has regulation for total aflatoxin only. FAO has expressed need for regulatory authorities in countries, where total aflatoxin regulatory limits apply, to inspect available data

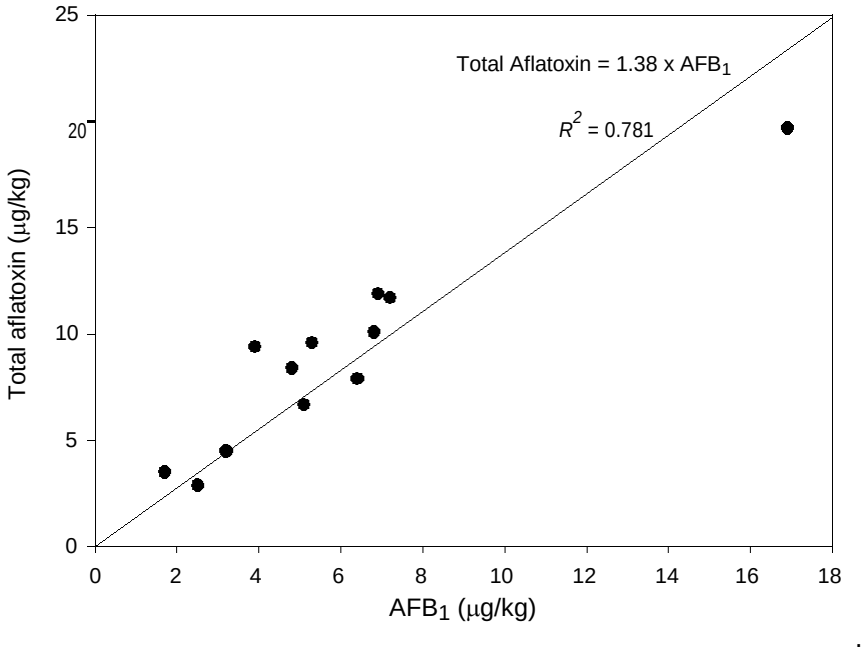

Figure 4. Relationship between AFB_1 and total aflatoxin in stored maize samples.

Table 3. A summary of average reduction (%) in AFB_1 by a single process (dry matter basis). The AFB_1 levels and reduction percentages displayed are means of 10 replicates. Different letters within a row indicate that means are significantly ($p < 0.05$) different according to Tukey's HSD test.

Process	Initial AFB_1 (µg/kg)	Final AFB_1 (µg/kg)	AFB_1 reduction (%)
Soaking grit for 72 h and washing	33.9± 18.9	7.2± 5.3	80.9 ±5.3$_a$
Soaking grit for 48 h and washing	33.9± 18.9	8.6± 5.5	75.4 ±3.5$_{ab}$
Soaking grit for 24 h and washing	33.9± 18.9	9.5 ± 6.1	72.4 ±5.4$_b$
Dehulling maize	48.4± 26.8	33.9± 18.9	29.3 ±5.4$_c$
Sun drying whole meal flour (*mgaiwa*)	48.4± 26.8	42.1 ± 22.4	11.7 ±3.2$_d$
Sun drying non soaked dehulled flour (*gramil*)	33.9± 18.9	29.4± 15.3	11.4 ±4.2$_d$
Sun drying 72 h soaked grit flour (*woyera*)	9.5± 6.1	6.4 ±5.3	11.4 ±3.3$_d$
Sun drying 24 h soaked grit flour (*woyera*)	9.5± 6.1	8.6± 5.4	9.4 ± 3.6$_d$

on aflatoxins and determine the ratio of AFB_1 to total aflatoxin in order to protect consumers (FAO, 2004). Due to the toxic nature of AFB_1, setting a separate (lower) level for AFB_1 offers an extra assurance for public safety. This study therefore sets the right direction for AFB_1 regulation in Malawi.

In the survey conducted on the occurrence of AFB_1 in flour, 37 and 27% of *mgaiwa* and *woyera* flour, respec-tively, presented a detectable AFB_1 level of contamination (Figure 5). The highest contaminated *mgaiwa* sample had 7.1 µg/kg while the highest contaminated *woyera* flour sample had 2.3 µg AFB_1/kg. The average AFB_1 content in *mgaiwa* samples was 1.00 ± 1.60 µg/kg, which was significantly ($p < 0.05$) higher than in *woyera* flour (0.38 ± 0.69 µg/kg). The low AFB_1 levels in flour samples compared to levels in maize samples point towards the efficiency of the flour processing methods in reducing aflatoxins.

Effect of maize flour production procedures on AFB_1 level in flour

Aflatoxin concentration in the artificially contaminated maize ranged from 8.9 to 87.1 µg/kg. Our results indicate that dehulling of maize, soaking of dehulled maize and drying reduced AFB_1 significantly in the order: soaking of dehulled maize > dehulling of maize > sun drying (Table 3). On average 29.3 ± 5.4% (range 16.6 – 34.9%) of the AFB_1 in the original maize was removed by simple bran removal (dehulling) (Figure 6). The bran represented 26.3% of the original mass of raw maize and contained

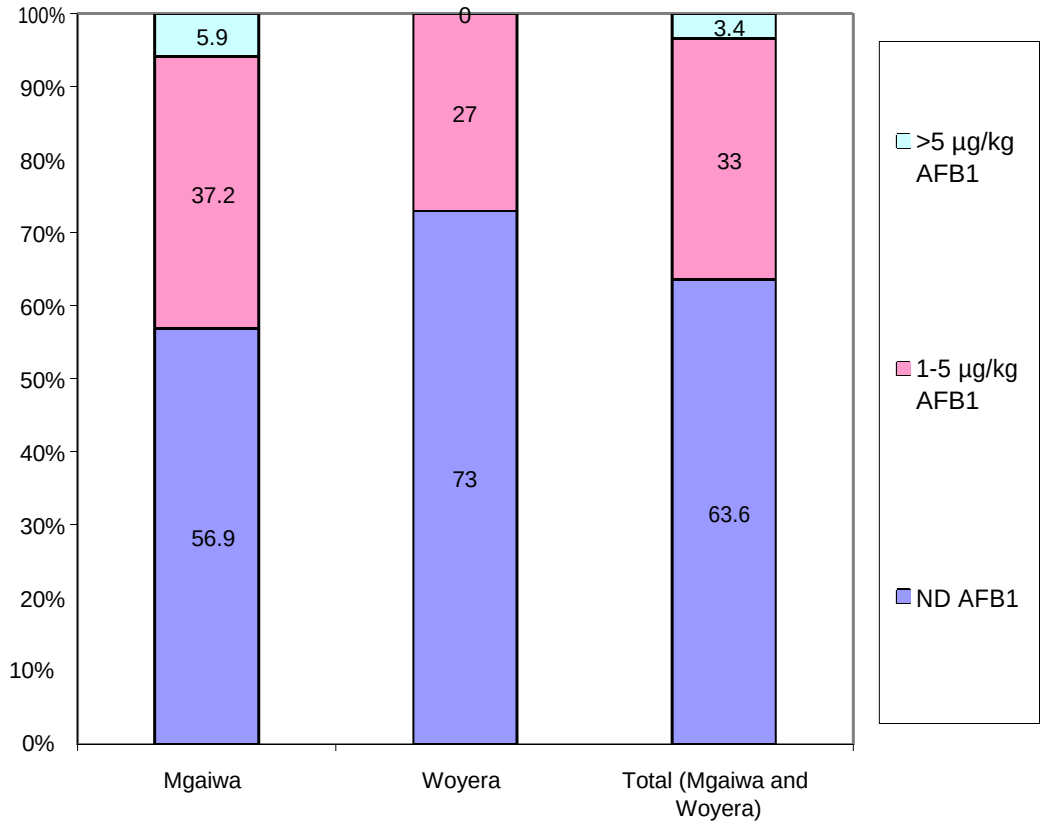

Figure 5. Incidence (%) of aflatoxin contamination in flour samples collected from hammer mills in *Mpingu* EPA, Lilongwe, Malawi.

on average 87.6 ± 7.8% more AFB_1 than the *mgaiwa* flour (Figure 6 (*a*)). Our results are in general agreement with previous studies (Bennet and Anderson, 1978; Njapau et al*.,* 1998). Bennet and Anderson (1978) observed slightly higher (30 - 38%) AFB_1 reduction through dehulling of maize.

Siwela et al. (2005) reported as high as 92% reduction of total aflatoxin through dehulling of maize. Ideally, the dehulling process aims at removing all the bran leaving only the endosperm, but this is only achievable in sophisticated commercial systems. The difference in the results could be due to differences in the efficiencies of dehullers used and extent of fungal and toxin penetration in the grains. We did not obtain significant relationship between initial AFB_1 concentration and AFB_1 percentage reduction caused by dehulling process (Figure 7 *(a)* and Table 4). This is because the dehulling process involves only mechanical removal of bran, the rate of which is independent of the aflatoxin level of the maize. In Malawi, the bran is widely used as animal feed and in times of food shortage people collect it from maize mills and prepare *nsima* from the resulting bran flour.

In these cases, both animals and humans are therefore at risk of aflatoxin toxicity. Further, risk-benefit analysis studies need to be carried out on aflatoxin decontamination through dehulling before it is extensively promoted as a precautionary measure against aflatoxin exposure as it is associated with nutrient loss. Moeser et al. (2002) observed the loss of neutral detergent fiber (NDF) (61%) and acid detergent fiber (ADF) (32%), lysine (33%) and all other essential amino acids except methionine and tryptophan, following dehulling and de-germing of maize.

Soaking and subsequent washing of dehulled maize removed substantial amounts of AFB_1 content. Soaking dehulled maize for 24, 48 and 72 h reduced AFB_1 content significantly ($p < 0.05$) by 72.4 ± 5.4, 75.4 ± 3.5 and 80.9 ± 5.3% respectively (Table 3 and Figure 6 *(c)*). This observation is in agreement with the findings of Bennet and Anderson (1978) and Se (2002) who reported a recovery of 39 - 42% in aflatoxins in steep water and 48% reduction in aflatoxin due to loss of steep water, respectively. Our results of the treatment experiments show no significant difference between soaking for 24 and 48 h and between 48 and 72 h on aflatoxin removal. However, soaking for 72 h yielded significantly ($p < 0.05$) higher AFB_1 reduction than soaking for 24 h (Table 3).

During soaking, pH dropped from 6.7 at the beginning of the soaking period to 5.8 after 24 h, 4.6 after 48 h and 4.3 after 72 h. The drop in pH may be attributed to microbial activity. We did not find a significant relationship between initial AFB_1 concentration and percentage AFB_1 reduction caused by the 24 and 48 h soaking processes,

Table 4. A summary of regression equations obtained using stepwise regression analysis using SPSS for Windows 12. The regressions equations are plotted together with experimental data in Figures 7 and 8.

Figure	Plot	Regression equation	Sign.	S.E	F	R^2	$R^2_{adjusted}$
Figure 7	Dehulling, Soaking for 24 and 48 h and sun drying *woyera* flour	No significant regression					
	Soaking for 72 h.	AFB_1 reduction (%) = -0.234$[AFB_1]_o$ + 88.81	0.002	3.005	19.599	0.71	0.67
	Sun drying *mgaiwa* flour	AFB_1 reduction (%) = 0.106$[AFB_1]_o$ + 6.56	0.001	1.543	30.345	0.791	0.765
	Sun drying *gramil* flour	AFB_1 reduction (%) = 0.197$[AFB_1]_o$ + 4.70	0.001	2.165	26.652	0.769	0.740
Figure 8	Sun drying *Mgaiwa* flour	log $rate_o$ = 1.344log $[AFB_1]_o$ – 2.15	<0.001	0.7767	279.702	0.972	0.969
	Sun drying *Gramil* flour	log $rate_o$ = 1.383log $[AFB_1]_o$ –2.18	<0.001	0.10262	159.052	0.952	0.946
	Sun drying *woyera* flour (24 h soaking)	log $rate_o$ = 1.176 log $[AFB_1]_o$ –1.87	<0.001	0.19411	35.846	0.818	0.795
	Sun drying *woyera* flour (72 h soaking)	log $rate_o$ = 1.023log $[AFB_1]_o$ –2.11	<0.001	0.13657	86.202	0.915	0.904

but for the 72 h soaking process (Figure 7 *(b)* and Table 4). This suggests that the microorganisms, although present, may not have been involved in reduction of AFB_1 during the 24 and 48 h soaking processes. On the other hand, the 72 h soaking process may have provided sufficient time for degradation of AFB_1 by microbial activity, as a certain time may be needed (lag phase) for cells to adjust to the environment. The reduction of AFB_1 may therefore be attributable to partial solubility of AFB_1 in water (Grant and Phillips, 1998) and microbial activity, which leads to reduced pH.

In this study, a difference in soaking time of 48 h was required to obtain a significant difference in AFB_1 reduction (24 and 72 h soaking periods). This period may have allowed both more AFB_1 to dissolve in the steep-water and for microbial action, hence more AFB_1 removal during the draining of the steep water.

We established, through interviews during the study, that people use steep-water for cooking sour porridge, especially for patients. The steep-water would be a significant source of dietary AFB_1 exposure. Further, interviews conducted with maize mill operators at two maize mills located in *Biwi* and *Area 18* townships (Lilongwe City) revealed that the majority of the urban dwellers prefer preparing *gramil* to the *woyera* flour.

Sun drying for 4.5 h achieved mean AFB_1 reduction of 11.7 ± 3.2% for *mgaiwa*, 11.4 ± 4.2% for *gramil*, 11.4 ± 3.3% for *woyera* flour (72 h soaking) and 9.4 ± 3.6 % for *woyera* (24 h soaking), without significant differences between the flour types (Table 3 and Figure 6 *(d)*). The AFB_1 percentage reduction achieved through sun drying increased linearly with initial AFB_1 content in *gramil* (r^2 = 0.79) and *mgaiwa* (r^2 = 0.77) (Figure 7 *(d)* and Table 4). However, we did not find a significant relationship for sun-drying *woyera* flour (Figure 7 *(c)* and Table 4) probably due to low initial AFB_1 content and high moisture content. The experimental procedure for the sun-drying processes entailed exposing flour samples with different initial AFB_1 concentration to the sun for similar periods (4.5 h), corresponding to varying initial concentration of AFB_1 in flour samples, keeping the reaction time constant at 4.5 h. We therefore applied the method of initial rates (incorporating the isolation method) to determine the order of reaction for the reduction of AFB_1 with respect to AFB_1 through the sun-drying process. Firstly, we calculated the rates of reactions from the experimental data using:

$$\text{rate}\ (\mu\text{g/kg hr}) = \frac{[\text{AFB}_1]_o - [\text{AFB}_1]_{4.5hr}}{4.5hr} \quad (3)$$

where $[AFB_1]_o$ and $[AFB_1]_{4.5hr}$ denote the initial and final concentration of AFB_1 in the flour sample. The empirical initial rate law for the conversion of AFB_1 may be written as:

$$\text{rate}_o = k\,[\text{AFB}_1]_o^{\alpha} \prod_i^N [X]_o^{\gamma_i} \quad (4)$$

where k is the rate constant, α is the order of reaction with respect to AFB_1, $[AFB_1]_o$ is the initial concentration of AFB_1 in the flour sample, $[X]_{o,i}$ is the initial concentration of other species i that may be present to react with AFB_1 and γ_i is the order of reaction with respect to species i.

Supposing that the concentration of the other species is in excess, (i.e. AFB_1 is isolated) and hence their concentrations do not vary significantly with time, equation 5 leads to:

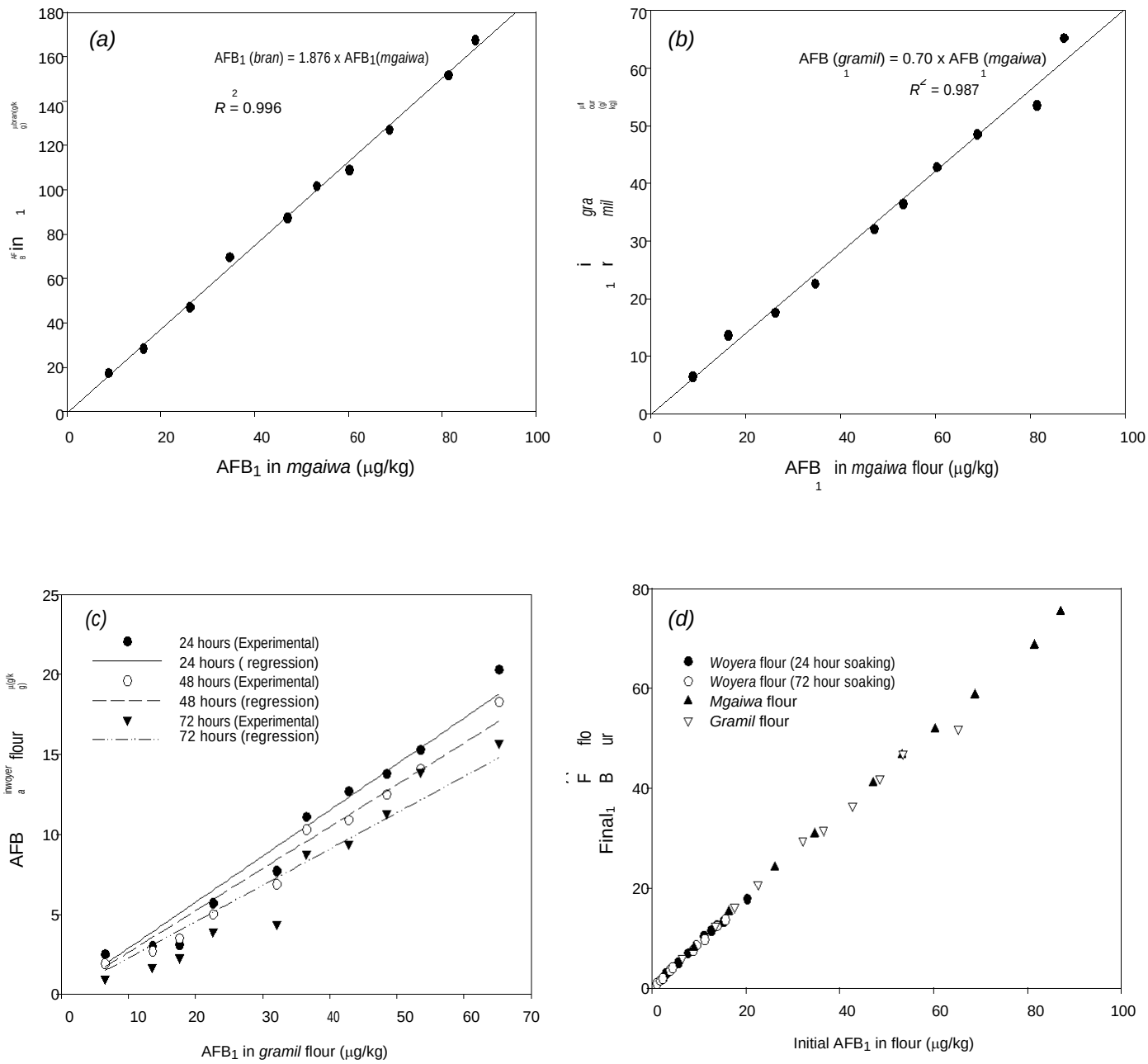

Figure 6. Plots of initial and final AFB_1 content in different flour products produced using traditional methods outlined in Figure 2, depicting the efficiency of the methods in AFB_1 reduction. Dehulling (a) and (b); soaking for 24, 48 and 72 h *(c)*; and sun-drying *(d)*. For all plots, points are experimental data and lines are regression lines. For *(c)*, 24 h soaking: AFB_1(*woyera*) = 0.288 x AFB_1 (*gramil*), R^2 = 0.966; 48 h soaking: AFB_1(*woyera*) = 0.261 x AFB_1 (*gramil*), R^2 = 0.972; 72 hour soaking: AFB_1(*woyera*) = 0.227 x AFB_1 (*gramil*), R^2 = 0.924.

$$\log \text{rate}_o = \alpha \log[\text{AFB}_1]_o + \log K \qquad (4)$$

where log *K* is a constant given by

$$\log K = \log k + \log \prod_i^N [X]_o^{\gamma_i}$$

To find the order of reaction, we plotted log $rate_o$ as a function of log $[AFB_1]_o$ (equation 5). Further, we applied stepwise linear regression analysis using SPSS for Windows 12.0 to the calculated pairs of log $rate_o$ and log $[AFB_1]_o$. We found strong and significant linear regres-sions expressing log $rate_o$ as a function of log $[AFB_1]_o$ for all the flours, with $\alpha \approx 1$, indicating a first order reaction in AFB_1 concentration (Table 4 and Figure 8). We note that, in Malawian villages, *mgaiwa* and *gramil*

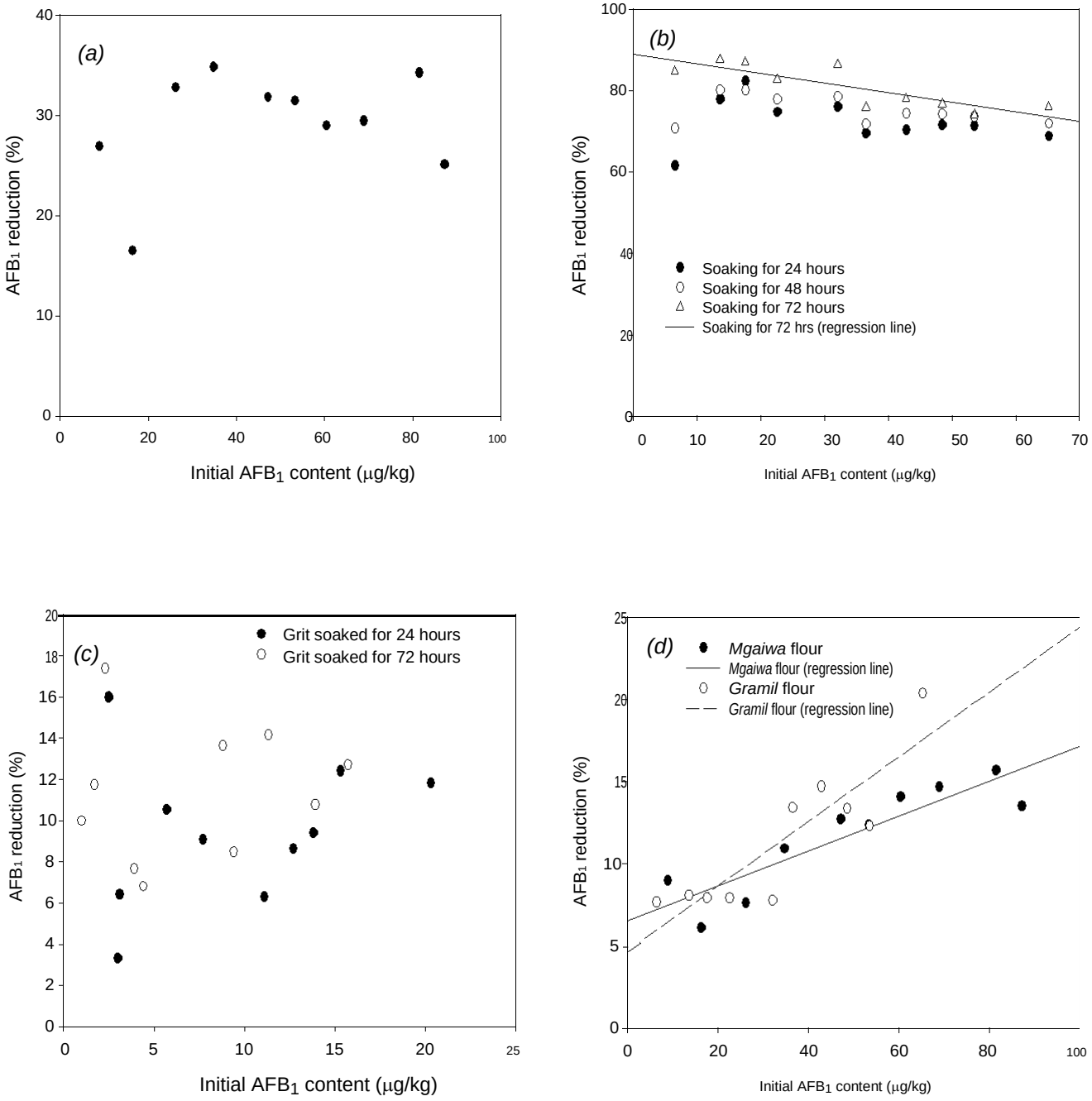

Figure 7. Variation of percentage AFB_1 reduction with initial AFB_1 content. Dehulling (a); soaking for 24, 48 and 72 h (b); and sun drying *woyera* flour produced after 24 and 72 h soaking periods (c) and sun drying *mgaiwa* and *gramil* flour (d). For all plots, points represent experimental data and lines are plots of significant linear regression equations summarized in Table 4.

are not sun-dried because they already contain low moisture levels, which are less likely to support microbial growth to spoil the flour.

When the combined effect of the process is considered, the final AFB_1 level in flour samples from our experiments compare very well with those obtain in the maize flour survey. The maximum AFB_1 reduction (88.1 ± 3.1%) was achieved from combined processes of dehulling, soaking (72 h) and sun drying of flour (4.5 h) (Table 2 and Figure 9).

Conclusion

This study has shown that aflatoxin contamination in stored maize is wide spread in *Mpingu* EPA, reflecting

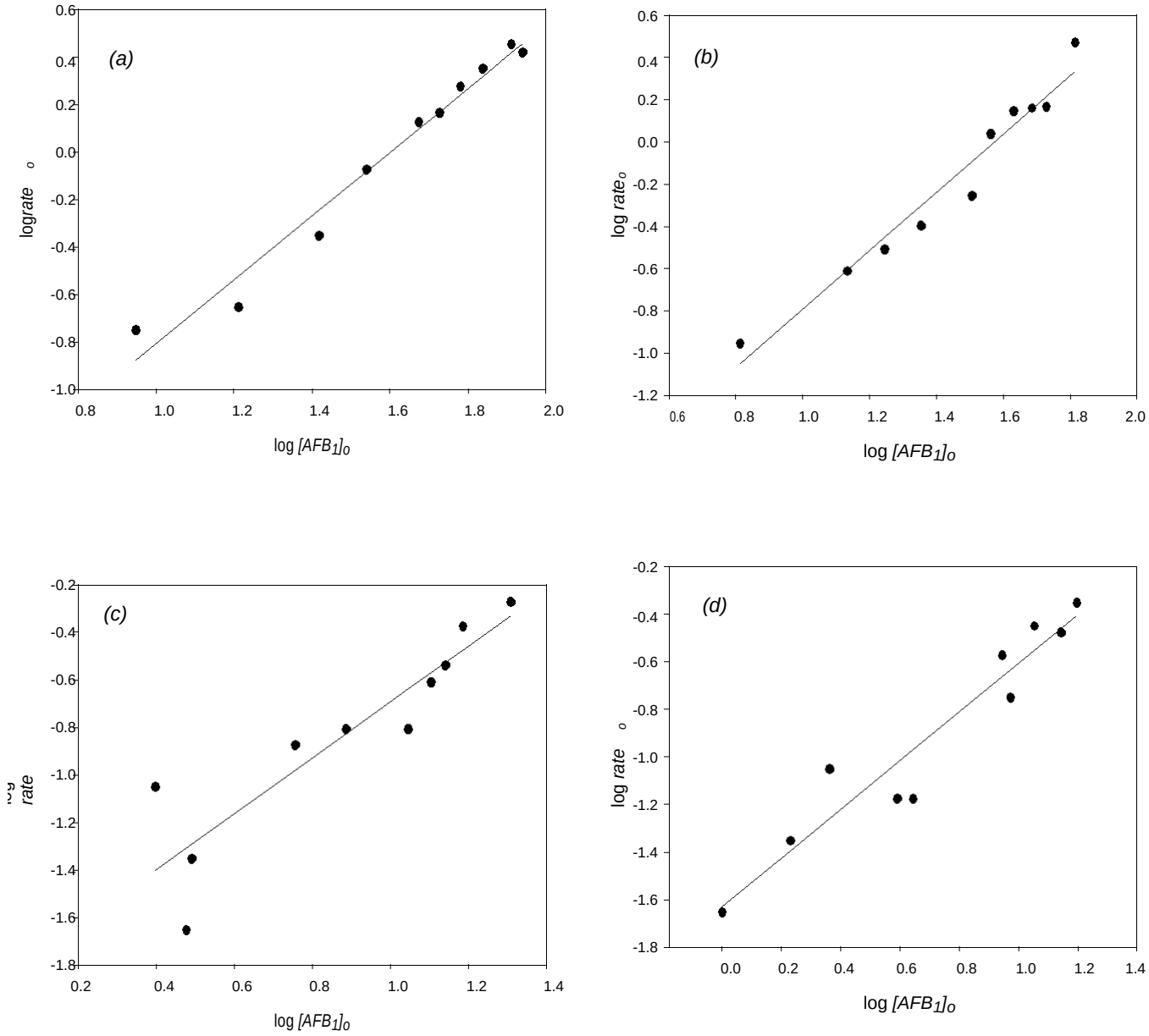

Figure 8. Plots of log initial rate and log initial AFB_1 content for sun drying of mgaiwa flour (a), gramil flour (b), woyera flour (24 h soaking) (c), and woyera flour (72 h soaking) (d). The dots represent data from experiment, whilst the straight lines are plots of significant linear regression equations summarized in Table 4.

wide spread contamination in Malawi. Further, we have also shown that despite the prevalence of aflatoxin contamination in stored maize, the traditional methods of maize flour production are effective in reducing AFB_1 content in the final flour and hence in *nsima*, thick porridge made from the flour. The processes of dehulling, soaking, milling and sun drying the resulting flour achieved a combined AFB_1 reduction of 88.1%. The efficiencies of the individual processes were in the order: soaking and drying > dehulling > sun drying. However, the aflatoxin reduction during processing may not necessarily translate into low exposure and risk due to high consumption.

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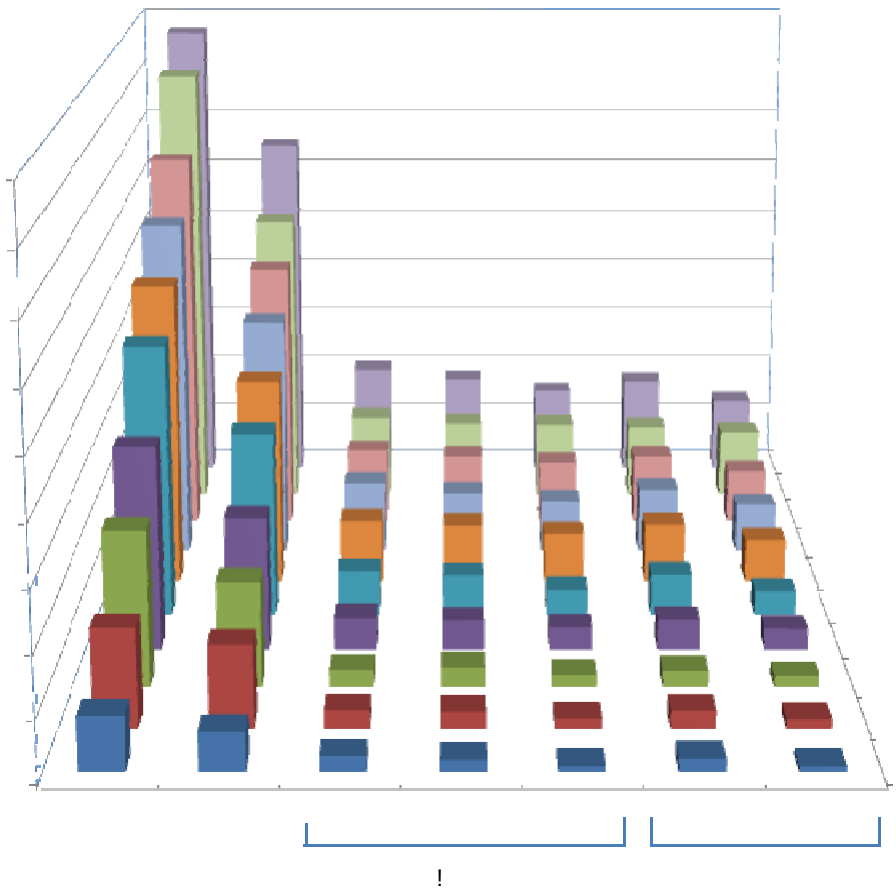

Figure 9. Variations in AFB_1 content during processing for the production of *woyera* flour using the village processing techniques. Letters (*a, b … j*) denote different levels of contamination in the starting maize.

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