Maize meal slurry mixing: an economical recipe for precise aflatoxin quantitation

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Abstract

The laboratory sample preparation for mycotoxin determination in cereals, often overlooked among sampling plans and analytical methods, was further studied. The precision of aflatoxin analysis in comminuted maize samples using 25 g slurry (prepared from 250 g test portion of comminuted maize, water/matrix (1+1, v/w)) and 12.5 g dry grind test portion were compared against the conventional 50 g dry grind test portion through replicated (10) Aflatest® immunoaffinity fluorometric tests of naturally contaminated samples with aflatoxin concentration ranging from 4.9 to 81.7 µg/kg. The overall mean aflatoxin concentration obtained from the 10 different samples tested using 12.5 g and 50.0 g dry grind procedures was 12% significantly ($P < 0.05$) lower (poorer) compared to 25 g slurry. The sample preparation plus analytical variance associated with testing 25.0 g slurry, 50.0 g dry grind and 12.5 g dry grind test portions were in the ratio of 1:5:15, respectively.

Keywords: dry grind, slurry mixing, sample preparation, aflatoxin analysis, maize, precision, recovery

1. Introduction

Aflatoxin contamination in grains, particularly maize, remains a major challenge globally (Battilani *et al.*, 2016). Aflatoxins are toxic metabolites produced by a variety of *Aspergillus* species, notably *Aspergillus flavus* and *Aspergillus parasiticus* and are capable of causing cancer, mutagenesis, teratogenesis immunosuppression and nutrition interference in animal species and humans (Williams *et al.*, 2004). Consequently, about 100 countries have established strict regulatory limits for aflatoxin in food to as low as 100 ng/kg in baby food to reduce dietary intake (EC, 2010; FAO, 2003).

These control activities require measurement methods that can accurately and precisely measure the true aflatoxin concentration in food and feed stuff. Typically, aflatoxin test procedure in grains consists of 3 steps: (1) the sampling step, where a random sample (laboratory sample) is taken from the lot; (2) the sample preparation step, where an

entire laboratory sample is comminuted in a mill or grinder and a subsample (called a test portion) is drawn from the comminuted laboratory sample. Grinding and subsampling are collectively called the sample preparation step; (3) the analytical step, where aflatoxin in the test portion is solvent extracted, purified, and quantitated (Whitaker *et al.*, 2010). Mostly, aflatoxin quantitation involves chromatographic technique such as thin-layer chromatography (TLC), gas chromatography (GC), or high-performance liquid chromatography (HPLC) coupled with a photo diode array, UV-Vis or fluorescent detector, or liquid chromatography with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) (Berthiller *et al.*, 2017; Shephard, 2016). However, recently fluorometer based detection of aflatoxin has become popular (Magamba *et al.*, 2017; Manzoza *et al.* 2017; Nishimwe *et al.*, 2017; Schwartzbord and Brown, 2015).

The variability associated with each of the 3 steps contributes to the total variability (random errors)

associated with the aflatoxin test procedure. However, studies on a wide variety of agricultural products indicate that the sampling step is generally the largest source of variability followed by sample preparation and analysis in that decreasing order (Johansson *et al.*, 2000a; Ozer *et al.*, 2017; Whitaker, 2006; Whitaker *et al.*, 1974). Although it is impossible to completely eliminate sampling variability, several workers have developed sampling plans that reduce random error to levels considered to be acceptable by those responsible for interpreting and reporting the surveillance data (Johansson *et al.*, 2000a; Whitaker, 2003). Similarly, many analytical methods have been validated and used for the analysis of aflatoxins (Berthiller *et al.*, 2017; Shephard, 2016; Turner *et al.*, 2015).

While great efforts have been made to establish reliable sampling plans and analytical methods with acceptable performance criteria, little attention has been paid to the sample preparation phase. Typically, during sample preparation, a laboratory sample of several kilograms is dry ground and the comminuted sample is thoroughly mixed to try and approach complete homogenisation. A small subsample, called a test portion, is removed from the comminuted sample for extraction of the mycotoxin to minimise costs associated with extraction solvents. However, for a given particle size distribution, test portion size has an effect on the variance associated with the sample preparation step. Whitaker *et al.* (1994) found that halving a size of test portion increased variance by a factor of 2. For example, variance associated with a 10 g dry grind test portion from a Romer mill was found to be five times greater than the variance associated with a 50 g dry grind test portion (Whitaker, 2006). From a series of studies, Whitaker and his group, concluded that analysing a 50 g test portion (dry grind) taken from a laboratory sample comminuted in a Romer mill is the minimum that should be employed with reasonably acceptable variance. Consequently, analysis of 25 to 50 g dry grind test portion were typical test portion sizes recommended for extraction when the laboratory sample is dry-ground in commercially available mills, such as the Romer mill (Bansal *et al.*, 2011; Firdous *et al.*, 2014; Garrido *et al.*, 2012; Lewis *et al.*, 2005). However, the cost associated with analysis of the larger test portion sizes (25 to 50 g dry grind test portions) is still considered to be high particularly in resource constrained settings including Africa where extraction solvents are mostly imported from overseas.

Studies on several agricultural products, mostly oil seeds have shown that slurry-mixing approaches can potentially reduce sample preparation variance (Schatzki and Toyofuku, 2003; Spanjer *et al.*, 2006; Velasco and Morris, 1976; Whitaker *et al.*, 1980, 1994). However, it is not very clear if such reduction in variance could be leveraged to successfully reduce test portion size further from the 50 g. Therefore, this study was performed to explore the most economical

(minimum) test portion mass of a maize slurry that could practically be used in the analysis of aflatoxin using VICAM Aflatest® (Vicam, Milford, MA, USA) immunoaffinity fluorometric analysis without compromising precision with reference to analysis of conventional 50 g dry grind test portion. Drawing from the above, we derived the following hypothesis: 'better precision of aflatoxin quantification in maize meal is achievable by slurry mixing a large test portion and analysing a small fraction of it than using the conventional 50 g dry grind'. Further, the variance associated with 25 g slurry (12.5 g maize), 12.5 g dry grind and the conventional 50 g dry grind procedures and the effect of matrix/water ratio in a slurry on the recovery and precision were studied and discussed.

2. Materials and methods

Maize sample and preparation

Ten naturally contaminated maize samples (2 kg each) varying in aflatoxin concentration were bought from Chapananga market in Chikwawa district (Malawi) and separately ground to pass sieve # 20 (opening size = 0.85 mm) using Romer Labs Series II® mill (Romer Labs, Inc., Union, MO, USA). The choice of Romer mill was based on the fact that it is the most frequently used mill for quality control in mycotoxin analysis, providing both grinding and subsampling of grains (Lippolis *et al.*, 2012; Nowicki and Roscoe, 2010; Spanjer *et al.*, 2006; Whitaker *et al.*, 2000). Two kilograms (2 kg) of each comminuted sample was then apportioned into 1 kg, 500 g and two 250 g sub-samples using a riffle-divider (Humboldt Testing Equipment, Norridge, IL, USA).

Study design

Four experiments were performed to achieve the study objective. The first experiment was performed to determine the minimum test portion mass of a slurry that could practically be employed to analyse aflatoxin in a maize sample based on an equivalent Aflatest VICAM dry grind-based extraction procedure. In this procedure, a 10 ml volume filtered extract is required for the analysis.

Five different slurry aliquots of 30, 25, 20, 15 and 10 g prepared by mixing water/matrix (1+1, v/w) ratio were tried. The water matrix ratio of 1+1 was used to match the Aflatest VICAM dry grind-based extraction procedure. After blending the maize/water mixture for 3 min, the slurry was blended at high speed for 2 min following the addition of an appropriate volume of methanol aimed at achieving a mixture of water/matrix/methanol (1+1+3, v/w/v) ratio. Finally, the volume of filtrate (passed through fluted paper) obtained at the end of 2 min was recorded.

Informed by results of the first experiment, the second experiment was performed to compare the combined sample preparation plus analytical variance (s_{spa}^2) associated with aflatoxin analysis of 12.5 g dry grind, 50 g dry grind and 25 g slurry [water/matrix (1+1, v/w)] test portions taken from naturally contaminated maize samples (Figure 1). The combined sample preparation plus analytical variance is the sum of the sample preparation variance (s_{sp}^2) plus the analytical variance (s_a^2).

$$s_{spa}^2 = s_{sp}^2 + s_a^2 \quad (1)$$

The maize slurry was prepared by mixing 250 g of the ground maize with 250 ml distilled water (water/matrix ratio of 1+1, v/w) and blended at high speed for 3 min. It is important to note that the 12.5 g dry grind and 25 g slurry only differ in one respect, the sequence in which the extraction fluid is added. In dry grind treatment, the steps are (1) weigh 12.5 g test portion, (2) add 1.25 g of NaCl, (3) add 50 ml of blend of 12.5 ml of water and 37.5 ml of MeOH, (4) blend for 2 min in a blender, and (5) filter. For the slurry treatment, the sequence is (1) weigh 250 g test portion of dry ground maize and add 250 ml water, (2) blend for 3 min to make slurry, (3) weigh out 25 g of slurry, (4) add 1.25 g NaCl, (5) add 37.5 ml MeOH, (6) blend for 2 min, and (7) filter.

For each of the 10 samples, 10 replicates (Figure 1) from each of the three test portion treatments were measured

for aflatoxin to elucidate the combined sample preparation plus analytical variance (s_{spa}^2). Additionally, for each of the 10 samples, 10 aliquots from a single 50 g dry grind sample extract were analysed to elucidate the analytical variance (s_a^2) associated with the Aflatest immunoaffinity fluorometric analytical procedure (Figure 1). It is assumed that the analytical variance is the same for all three test portion treatments.

The third experiment was performed to determine the effect of duration of blending on extraction efficiency. In this regard, the efficacy of extracting aflatoxin from a 50 g dry grind test portion (in triplicate) for 2- and 5-min blend times were compared. In this experiment, the 2-min blending represents the common practice as outlined in the VICAM Aflatoxin Test Manual (VICAM, 2014). On the other hand, the 5-min blending represents the sum of blending times involved in slurry preparation (3 min) and the conventional methanol/water extraction (2 min).

The fourth experiment was performed to determine the effect of water/matrix ratio on the precision of scooping maize solids during weighing of test portions. In this regard, three water/matrix ratios of 2/5, 1/1 and 8/5 were evaluated based on common practice and insights from previous experiments (Cole and Dorner, 1994; Stroka, 2000). The slurries were prepared by blending the water/matrix mixtures for 3 min as described earlier. Subsequently, 25 g test portions (6 replicates) were scooped into pre-weighed dishes using spatula and dried in an oven at 120 °C for 4 h. The mass of the dried test portion was recorded after cooling the aliquot in a desiccator and the percent error was calculated as a measurement of the discrepancy between an observed (experimental) mass and expected mass using the following formula:

$$\text{Percent error} = \frac{(\text{observed mass} - \text{expected mass}) \times 100}{\text{expected mass}}$$

Where the expected mass refers to anticipated theoretical mass of solids based on the water-solid combination in a slurry adjusted for 10% intrinsic moisture loss incurred during drying assay.

Aflatoxin determination

Extraction and clean-up of aflatoxin from maize test portions was performed using a modified version of the manufacturer's instruction for the Aflatest immuno-affinity procedures for popcorn (VICAM, 2014). Aflatoxin was extracted from the dry-ground test portions (50 g maize + 5 g NaCl and 12.5 g maize + 1.25 g NaCl) with 200 ml and 50 ml of methanol/water (150+50 v/v) and (37.5+12.5 v/v), respectively, and blended at high speed for 2 min. Similarly, extraction of aflatoxin from 25 g slurry-mixing portions involved 37.5 ml HPLC grade methanol blend at high speed

Figure 1. Scheme of sample preparation (second experiment).

for 2 min. The triturated mixture was filtered through fluted paper to remove particulate matter. A 10 ml volume of the filtered extract was diluted with 40 ml distilled water, mixed and filtered through a glass-fibre filter paper. A 20 ml (1 g sample equivalent) of the diluted extract was passed through Aflatest immuno-affinity column at a flow rate of about 1-2 drops/s. The column was washed twice with (10 ml) distilled water at a rate of (2 drops/s) to remove maize intrinsic compounds and finally the aflatoxins were selectively eluted with 1 ml of 100% HPLC grade methanol into a glass cuvette. One ml of Aflatest developer (developer/ water of 9+1, v/v) was added to elute in the cuvette and mixed using vortex before reading the sample in a calibrated VICAM Series-4EX Fluorometer.

Recovery experiments

Reference maize sample (R-Biopharm AG, Darmstadt, Germany) at certified concentration for aflatoxin = 24.4 µg/kg was analysed using 12.5 g dry grind, 50 g dry grind and 25 g slurry (12.5 g maize) approaches to determine recoveries and relative standard deviation (RSD) corresponding to recovery values. Analysis of the reference material was done in four replicates except for the 50 g dry grind which had three replicates. Further, repeatability (RSD_r) (the repeatability CV) was evaluated by performing the assay on five replicates of naturally contaminated maize meal and performed by the single operator on the same day (intra-day precision) and during five consecutive days (inter-day precision). The results were expressed as the percentage of relative standard deviation of the measurements (%RSD).

Statistical analysis

Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS version 23 (IBM Corp, Armonk, NY, USA) were used for statistical analysis and data presentation. The differences among using 25 g slurry to 12.5 g and 50 g dry grind procedures were analysed by one-way ANOVA. Results showing significant differences were subjected to post-hoc Tukey's test with significance for $P < 0.05$.

3. Results

Filtrate volumes attainable from 30, 25, 20, 15, and 10 g of maize slurry

The filtrate volumes obtained from five different test portion sizes are presented in Supplementary Figure S1. As expected, the filtrate volumes decreased with a reduction in test portion (Filtrate = $0.602 \times \text{mass of slurry} - 1.7$; $R^2 = 0.9824$). Of the experimented test portions, the 25 g of slurry was found to be the minimum mass that constantly yielded sufficient volume (enough to practically pipette 10 ml) required for the Aflatest immunoaffinity fluorometric assay within 2 min filtration time. Consequently, the 25 g

slurry test portion (12.5 g water + 12.5 g maize) was used in subsequent experiments.

The effect of extraction blending time on aflatoxin recovery

The effect of extraction time on aflatoxin recovery was examined by comparing 2- and 5-min blending time with constant experimental conditions. Blending 50 g maize meal (in triplicate) with 200 ml methanol/water (150+50 v/v) for 2 and 5 min yielded 8.1 ± 1.7 and 7.8 ± 1.3 µg/kg, respectively, with no significant ($P < 0.05$) difference according to t-test.

Effect of water/maize ratio on the precision of scooping maize proportion (dry solids)

Results on effect of water/matrix (maize) ratio on the precision of scooping maize proportion (dry solids) during weighing of test portions are presented in Supplementary Table S1. Water/maize ratio had no effect on the precision of scooping maize proportion (dry solids) over the studied range (2/5-8/5). The percent errors associated with all the three water/matrix ratios (2/5, 1/1 and 8/5) were less than 3%.

Variability of aflatoxin results obtained with 12.5 g dry grind, 50 g dry grind and 25 g slurry preparation procedures

Aflatoxin analysis (10 replications) of 12.5 g dry grind, 50 g dry grind and 25 g slurry [water/matrix (1+1, v/w)] of ten different naturally contaminated maize samples is presented in Supplementary Table S2, with total aflatoxin concentrations ranging from 4.9 to 81.7 µg/kg. The combined sample preparation plus analytical variance (analytical variance reflects the VICAM Aflatest immunoaffinity fluorometric analysis) associated with aflatoxin testing using 12.5 g dry grind, 50 g dry grind and the 25 g slurry sample preparation procedures are displayed in Figure 2. Assuming a power function is appropriate (Johansson *et al.*, 2000b; Whitaker *et al.*, 1974), a regression analysis was run on the combined sample preparation plus analytical variance (s^2_{spa}) versus aflatoxin concentration (C) for each sample preparation procedure.

$$12.5 \text{ g dry grind: } s^2_{\text{spa}} = 0.9005C^{1.3625} \quad (2)$$

$$50 \text{ g dry grind: } s^2_{\text{spa}} = 0.135C^{1.595} \quad (3)$$

$$25 \text{ g slurry: } s^2_{\text{spa}} = 0.0611C^{1.3268} \quad (4)$$

The combined sample preparation plus analytical variance associated with 12.5 g dry grind sample preparation procedure ranged from 10.2 to 499.4 µg²/kg² (mean 160.1 µg²/kg²) while as for the 50 g dry grind and 25 g slurry ranged from 2.6 to 134.4 µg²/kg² (mean 55.6 µg²/kg²),

and 0.7 to 32.1 $\mu\text{g}^2/\text{kg}^2$ (mean 11.0 $\mu\text{g}^2/\text{kg}^2$), respectively (Figure 2; Supplementary Table S2). Therefore, the mean combined variance associated with testing 12.5 g dry grind, 50 g dry grind and 25 g slurry were in the ratio of 15:5:1, respectively. The variance was positively related with aflatoxin concentration. The functional relationship between combined variance associated with the three sample preparation approaches and aflatoxin concentration is described by equations that are shown in Figure 2. For all the three sample preparation approaches, the coefficient of determination for the regression equation was greater than 0.8 (Figure 2).

The analytical variances (s_a^2) associated with the Aflatest immunoaffinity fluorometric method used in the present study are plotted against aflatoxin concentration in Figure 3. Similar to trends observed with combined variance, the analytical variance appeared to be a function of aflatoxin concentration. From a regression analysis the analytical variance is:

$$s_a^2 = 0.0473C^{1.2743} \quad (5)$$

For aflatoxin concentration between 4.9 to 81.7 $\mu\text{g}/\text{kg}$, the Aflatest immunoaffinity fluorometric analytical variance ranged from 0.3 to 8.8 $\mu\text{g}^2/\text{kg}^2$ (mean 5 $\mu\text{g}^2/\text{kg}^2$). The ratio of the analytical variance to combined variance was generally small particularly for 12.5 g dry grind and the 50 g dry grind approaches. The ratios of analytical variance to combined variance arising from aflatoxin testing of the 10 samples using 12.5 g dry grind and the 50 g dry grind approaches ranged from 1.0 to 5.8% (mean $\pm$ standard deviation (SD), $3.9 \pm 1.5\%$) and 4.6–39.6% (mean $\pm$ SD, $12.9 \pm 9.7\%$). Strikingly, the ratio of analytical variance to combined variance was comparatively higher in 25 g slurry ranging from 28.2 to 74.3% (mean $\pm$ SD, 50.8 ± 14.8). In other words, the variance

arising from slurry mixing of 250 g Romer Mill dry ground maize and analysing an aliquot of 25 g slurry [water/matrix (1+1, v/m)] was so low and comparable or even less than the analytical variability.

The variances associated with sample preparation (s_{sp}^2) for the three test portion treatments can be determined by solving for s_{sp}^2 in Equation 1.

$$s_{sp}^2 = s_{spa}^2 - s_a^2 \quad (6)$$

Then s_{sp}^2 for the three sample prep procedures:

$$12.5 \text{ g dry grind: } s_{sp}^2 = 0.9005C^{1.3625} - 0.0473C^{1.2743} \quad (7)$$

$$50 \text{ g dry grind: } s_{sp}^2 = 0.135C^{1.595} - 0.0473C^{1.2743} \quad (8)$$

$$25 \text{ g slurry: } s_{sp}^2 = 0.0611C^{1.3268} - 0.0473C^{1.2743} \quad (9)$$

The coefficients of variation (CV) associated with aflatoxin test procedures involving 12.5 g, 50 g and 25 g slurry [water/

Figure 3. Analytical variance associated with VICAM Aflatest® immunoaffinity fluorometric method.

Figure 2. A plot of sample prep plus analytical variance against mean aflatoxin concentration showing difference in the variance among three sample preparation procedures.

matrix (1+1, v/w)] of maize test portions comminuted in the Romer mill, and analysing one aliquot by the Aflatest immunoaffinity fluorometric method are shown over a range of aflatoxin concentrations in Figure 4. Each CV represents the combined variability associated with sample preparation plus analytical steps. The mean CV% associated with the three different sample preparation approaches differed significantly ($P<0.05$). The overall mean CV% associated with 12.5 g dry grind, 50 g dry grind and the 25 g slurry were 33.6, 18.7 and 7.8%, respectively, which translate to the ratio of 4:2:1, respectively.

However, for all the three sample preparation procedures, as expected, the CV% values improved (decreased) with increasing aflatoxin concentration (Figure 4). It is worth noting that linear relationship between CVs and aflatoxin concentration, C , displayed in Figure 4 is also predictable from the variance equations ($CV=S/C$) where S is the standard deviation or square root of variance.

$$12.5 \text{ g dry grind: } CV_{spa} = 0.949C^{-0.3188} \quad (10)$$

$$50 \text{ g dry grind: } CV_{spa} = 0.3674C^{-0.2015} \quad (11)$$

$$25 \text{ g slurry: } CV_{spa} = 0.2472C^{-0.3366} \quad (12)$$

Further results on intra-day and inter-day repeatability (precision) (RSDr) for 50 g dry grind and 25 g slurry approaches are provided in Table 1. Similar to the trends observed during analysis of the 10 different samples (aflatoxin concentration varied from 4.9 to 81.7 $\mu\text{g/kg}$), the 25 g slurry procedure produced lower intra-day and inter-day RSDr values compared to 50 g dry grind approach.

Effect of slurry mixing on aflatoxin extraction efficiency

The overall mean aflatoxin concentration obtained from the 10 different samples tested using 12.5 g and 50 g dry grind procedures were found to be 12% significantly ($P<0.05$) lower (poorer) compared to the mean obtained by using 25 g slurry approach (Table 2). Likewise, lower extraction percentage/recovery (about 10% lower) was observed with 12.5 and 50 g dry grind approaches as compared to the 25 g slurry procedure when reference material with 24.4 $\mu\text{g/kg}$ aflatoxin concentration was analysed (Table 3). Lower aflatoxin recoveries for dry grind approaches are also seen in Table 1 displayed earlier.

Figure 4. Relationship between aflatoxin concentration (C) and coefficient of variation (CV) % for the 50 g dry grind, 12.5 g dry grind and 25 g slurry sample preparation procedures.

https://www.wageningenacademic.com/doi/pdf/10.3920/WMJ2018.2415 - Wednesday, April 26, 2023 5:19:20 AM - IP Address:54.198.187.58

Table 1. Intra-day and inter-day assay precision of aflatoxin results obtained with 50 g dry grind and 25 g slurry obtained from same sample.

Sample preparation approach	Intra-day precision ¹		Inter-day precision ²	
	Aflatoxin ³ (µg/kg)	RSDr%	Aflatoxin ³ (µg/kg)	RSDr%
50 g dry grind	55.0±7.6	13.9	58.6±9.0	15.4
25 g slurry	66.8±4.9	7.4	63.2±4.1	6.4

¹ Five replicates performed by same operator on same day.
² Five replicates performed by same operator during five consecutive days.
³ Results are mean ± standard deviation.

Table 2. Overall means for aflatoxin concentration obtained using the three sample preparation procedures.

Procedure	Mean ^{1,2}
12.5 g dry grind	39.09 ^a
50.0 g dry grind	39.06 ^a
25.0 g slurry (12.5 g maize)	44.84 ^b

¹ The overall mean represents averages of aflatoxin concentration from all ten samples analysed.
² Values with a different superscript letter are significantly different ($P<0.05$).

Table 3. Means, relative standard deviation (RSD) and extraction percentage for reference material (24.4 µg/kg aflatoxin) analysed using three different sample preparation procedures.

Sample preparation procedure	Mean (n=4)	RSD %	Extraction %
12.5 g dry grind	21.4	5.0	87.5
50.0 g dry grind	21.7	4.7	88.9
25.0 g slurry (12.5 g maize)	23.9	2.2	97.8

4. Discussion

Improvements in sampling and sample preparation methods used to determine mycotoxin content in food continues to be a high priority among regulatory agencies, international organisations and commodity industries worldwide (Berthiller *et al.*, 2017). The present study has broadly explored the economical (minimum) test portion mass of a maize slurry in terms of methanol requirement that could practically be used to analyse aflatoxin without compromising recovery and precision with reference to analysis of conventional 50 g dry grind test portion. Furthermore, the efficiency of extraction of

aflatoxin for the two approached have been compared. The results demonstrate supremacy of 25 g water slurry mixing procedure over the 50 g dry grind procedure as regard to recovery and precision. The current exploration focused on Aflatest immunoaffinity fluorometric assay, however, the findings have wide applications and could significantly contribute to improvement of accuracy of mycotoxin quantitation worldwide.

Unlike previous studies which either investigated the effect of test portion mass reduction (Whitaker, 2006; Whitaker *et al.*, 1996) or slurry mixing (Spanjer *et al.*, 2006; Velasco and Morris, 1976) on recovery and precision, the present study uniquely investigated both variables simultaneously thus enabling direct comparison of these effects particularly because 25 g slurry comprised 12.5 g dry maize mass. The variance ratio of 15:5:1 for 12.5 g dry grind: 50 g dry grind: 25 g slurry mixing observed in the present study clearly indicate that slurring 250 g and testing 25 g slurry mass (which represents 12.5 g maize meal) has a greater (factor of 15 derived from 15:1) effect on improving precision than increasing test portion mass from 12.5 g dry grind to 50 g dry grind (factor of 3 derived from 15:5). Conversely reducing mass of test portion from a conventional 50 g dry grind to 12.5 g, increased variance by a factor of 3 which is closer to the value (4) predicted by Whitaker (2006) and Whitaker *et al.* (1996). A ratio less than 4 is expected since s^2_{spa} for each of the three test portion treatments has an analytical variance (Equation 1) component in the ratio.

Paradoxically, recent years have witnessed a dramatic reduction in test portion mass selected from the dry grinding of a laboratory sample with most laboratories extracting mycotoxins from a test portion smaller than 10 g of dry grind (Matumba *et al.*, 2017). In particular, studies that quantified mycotoxin in maize meal in European laboratories and published during 1997-2006 and 2007-2016 in Mycotoxin Research Journal had median test portion mass of 15 g and 5 g, respectively. This trend is worrisome if one considers that CVs for a given fineness of grind increase

with regard to lower levels of aflatoxin contamination. Thus, for lots where the aflatoxin concentration is close to the permissible/legal limit (often set at very low level, see FAO, 2003), consumers may be placed at risk of exposure to hazardous aflatoxin levels and processors placed at the risk of falsely rejecting an otherwise acceptable lot particularly when a conventional mill with sieve openings of 0.85 mm is used. When recommending small test portion sizes (i.e. 10 g) for extraction, it is extremely important to make sure the particle size resulting from the comminution process is very small to compensate for the recommended small test portion size. This study demonstrated that using water-slurry methods provides an easy and low-cost approach to reducing maize particle size so that the smaller maize mass (12.5 g) can be used in the extraction process without increasing sample preparation variability.

The good precision of the 25 g slurry procedure witnessed in the present study is not surprising. Slurry mixing increases degree of sample comminution (reduces particle size) which in turn enhances homogeneity of the test sample portion (Whitaker and Johansson, 2005; Whitaker *et al.*, 1980). However, there were concerns that the homogeneity could be influenced by water/matrix ratio as it could affect efficiency of distribution of particles (homogeneity) and slurry stability (suspended solids separating from slurry water over a prolonged time period). To the contrary, the results from the scooping experiment performed over the water/matrix ratio 2/5–8/5 (Supplementary Table S2), show high stability as evidenced by good precision of scooping maize proportion (dry solids). These results demonstrate the robustness of slurry mixing used in this study.

High extraction efficiency of slurry mixing procedure observed in the present corroborates earlier studies. Velasco and Morris (1976) and Whitaker *et al.* (1980) obtained 32 and 33% higher aflatoxin recoveries in groundnuts, respectively, with slurry mixing than when dry grinding was employed. Later, Schatzki and Toyofuku (2003) also noted higher (32%) recoveries with slurry mixing approach. Several explanations are given for the higher aflatoxin extraction efficiencies. These include extraction time (slurring mixing + MeOH extraction) and possible swelling or an exchange of H-bonding between aflatoxin and the matrix which eventually improves release of aflatoxin from the matrix during extraction (Schatzki and Toyofuku, 2003; Spanjer *et al.*, 2006). However, our results showed no effect of blending time on the extraction efficiency and thus only support the assertion of swelling or an exchange of H-bonding between aflatoxin and the matrix.

Besides improving recovery and precision of aflatoxin quantitation, slurry mixing procedure could reduce respiratory aflatoxin exposure of laboratory personnel associated with dry milling (Selim *et al.*, 1998) and practically cut the cost associated with methanol usage by a

factor of 4 (from 160 mL to 40 mL/sample as demonstrated in the present study). This is a great saving considering the high cost of purchasing of methanol and that wastes have to be discharged according to various national regulations for chemical waste disposal. However, the adoption of slurry mixing procedure is not without costs. Slurry making brings along extra personnel time involved in washing the blender or mixing equipment and increased volume of waste requiring proper discharge. While, it is important to perform economic analysis of adopting slurry mixing procedure as regards to waste management on a case-to-case basis, an analysis of adopting slurry mixing procedure by a laboratory in the Netherlands showed that slurring-mixing had negligible additional costs (Spanjer *et al.*, 2006). Innovatively, the slurry could be utilised as feed stocks for magic toxin detoxifying insects, such as Black Soldier Fly (BSF, *Hermetia illucens*) (Bosch *et al.*, 2017) and produce high quality insect biomass for livestock feed (Wang and Shelomi, 2017). Moreover, the waste from the BSF is considered high quality fertiliser (Sheppard *et al.*, 1994).

5. Conclusions

Sample preparation for mycotoxin quantification in maize meal, often overlooked among sampling plans and analytical methods, has been further studied by systematically comparing dry grind with slurry mixing procedure, for the determination of aflatoxin in maize samples. The current results provide evidence supporting the hypothesis that better recovery and better precision of aflatoxin quantification in maize meal is achievable by slurry mixing a large test portion and analysing a small fraction of it than using the conventional 50 g dry grind. Considering that globally aflatoxin regulatory limits for maize meant for human consumption are set mostly at level equal or less than 10 µg/kg where coefficient of variation test results are greatest, it is to the advantage of regulatory laboratories that use conventional laboratory mills to adopt slurring-mixing procedure to minimise the misclassification of lots, both accepting bad lots and rejecting good lots.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/WMJ2018.2415>.

Figure S1. Relationship between mass of slurry test-portion and filtrate volume.

Table S1. Error associated with scooping 25 g test portion from slurry prepared with different water/ maize meal ratios.

Table S2. Aflatoxin concentration and variance in ten samples as measured with 50 g dry grind, 12.5 g dry grind and 25 g slurry procedures plus analytical performance.

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Conflict of interest

The authors have declared that there is no potential conflict of interest.

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