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Influence of Sample Size and Analytical Procedure on the Variance of Surface Mould Plate Counts of Maize Kernels

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Summary: The influence of sample size (a), sample replication (b), analytical procedure (c) and analysis replication (d) on the total variance of surface mould counts of maize kernels was investigated in a commercial lot. Analytical variance of a procedure (option A) involving serial dilution and plating of liquid shaken with kernels was lower than that of a procedure (option B) where liquid shaken with subsamples of ground maize was diluted and plated (coefficients of variation of 23.93 % and 45.36 % respectively). Increases of sample size, number of replicate samples, subsample size (where applicable), and number of replicate analyses all reduced the total variance, but sample replication resulted in stronger reduction of total variance than analysis replication.

Einfluß von Probengröße und analytischem Verfahren auf die Varianz bei der Bestimmung der Anzahl von Oberflächen-Schimmelpilzen auf Maiskörnern

Zusammenfassung: Der Einfluss von Probenmenge (a), Probenahmewiederholung (b), analytischem Verfahren (c) und Analysenwiederholung (d) auf die totale Varianz bei der Bestimmung der Anzahl von Oberflächen-Schimmelpilzen auf Maiskörnern wurde in einer kommerziellen Maisprobe (40 000 kg) untersucht. Die analytische Varianz des Analysenverfahrens (Option A), in dem die Körner mit Flüssigkeit geschüttelt, diese stufenweise verdünnt und auf Platten aufgegeben wurden, war kleiner als die des Verfahrens (Option B), in dem die Flüssigkeit mit einer Unterstichprobe von gemahlenen Maiskörnern geschüttelt, verdünnt und auf die Platten aufgetragen wurde (Variations-Koeffizienten 23,93 % und 45,36 %). Eine Vergrößerung der Probenmenge, Erhöhung der Anzahl der Wiederholungsproben, Vergrößerung der Unterstichprobe (wo angewendet) und Erhöhung der Anzahl der Analysenwiederholungen verringerten alle die totale Varianz; durch eine Wiederholung der Probenahme erreichte man jedoch eine stärkere Reduktion der totalen Varianz als durch eine Analysenwiederholung.

Introduction

Little is known about the distribution of mould propagules in lots of maize, or about the errors involved in sampling maize for microbiological determinations. However, investigations of the factors contributing to the variability of aflatoxin analyses in maize [1] show that sampling variances play a major role compared to the analytical variance of the final aflatoxin determination. Due to the uneven distribution of aflatoxin in maize lots, increased sample sizes reduced the sampling variance considerably [1]. For practical purposes, a sample size of 4.5 kg has been recommended for the purpose of mycotoxin analyses in maize [2]. Such samples are ground, and a subsample of the meal is used for further analysis.

Chemical analyses, e.g. aflatoxin determinations, are not particularly affected by such protocols involving grinding operations. However, with microbiological analyses specific difficulties arise since aseptic processing of samples requires disinfection of the sample mill between grinding of different samples.

For the enumeration of surface moulds on maize kernels by plate count method, two options exist as in

Figure 1: (a) a direct method involving shaking of maize kernels in a washing liquid, followed by decimal dilution and plating, and (b) a method including grinding of the kernels, shaking a subsample of meal with washing liquid, followed by decimal dilution and plating. Figure 1 also shows the components of the total variance of each option, viz. σ^2_s sampling variance; σ^2_{ss} subsampling variance (where applicable); and σ^2_a or $\sigma^2_{a'}$: analytical variance. Option A can be realised by extraction in conical flasks on a shaker, as was done for this investigation, but also the use of the Colworth "Stomacher" falls under the same principle. This method is relatively fast and omits the subsampling variance σ^2_{ss}, but the size of the kernel sample is limited by the size of (shaker flasks and) equipment. In this investigation, option A accomodated samples ⩽ 500 g. Option B, although not truly representative for surface populations and more cumbersome, can accomodate larger samples of maize kernels such as advocated elsewhere [2].
In order to compare the above options, some of the principles described by Whitaker *et al.* [1] were applied to estimate the variances involved in the enumeration of surface moulds in a lot of maize.

Materials and Methods

Sampling and Dividing
The sampled lot consisted of a railway waggon containing 40,000 kg of maize of good quality, assessed by conventional grading standards. A sublot of 65 kg was collected by taking quantities of approx. 325 g every 15 seconds from the stream during the entire off-loading period (40 min). For the purpose of this paper, it is assumed that the microbiological population of this sublot is representative of that of the lot.
Using a Decco cone divider (James M. Decker Co. Inc., Baltimore, USA) the sublot was divided by repetitive halvings into adequate number of samples of 2 kg, 1 kg, 500 g, 250 g, and 50 g sizes.

Mould plate count procedure
(Option A): X g sample of maize kernels was transferred under aseptic conditions into 2 · X ml sterile 0.1 % peptone water (P.W.) in a 4 · X ml size conical flask. Material adhering to the kernels was extracted by shaking 1 hour on an orbital shaker (r = 13 mm) at 200 rpm.
(Option B): The whole sample of kernels was ground with a Condux toothed-disc mill (Condux-Werk, Hanau, FRG) into a coarse meal (90 % passed a sieve of 0.76 mm aperture). After mixing the meal thoroughly, Y g of meal subsample was transferred under aseptic conditions into 5 · Y ml P.W. in a 20 · Y ml size conical flask. Shaking as in option A.
In all cases, the first decimal dilution was made by adding 10 ml extract to 90 ml P.W.; all subsequent dec. dilutions by 1 ml previous dilution + 9 ml P.W. Counting (pouring) plates were prepared in duplicate with 2 ml of appropriate dilutions each, using malt extract (2 %) agar to which 30 mg/l filtersterilized tetracyclin was added prior to pouring. Incubation was at 28°C for 5 days. Plates containing 4-26 mould colonies were used for calculations.

Analytical variances σ^2_a and $\sigma^2_{a'}$:
σ^2_a *(Option A)*: Ten samples of 50 g kernels were treated as in mould plate count procedure option A. 50 ml from each of the ten obtained extracts were pooled. From this pool, 10 separate decimal dilution and plate count series were prepared.
$\sigma^2_{a'}$, *(Option B):* Ten samples of 10 g meal were treated as in option B. 25 ml from each of the obtained extracts were pooled and ten separate decimal dilution and plate count series prepared.

Combined sampling and analytical variances σ^2_{s+a} *resp.* $\sigma^2_{s+a'}$:
σ^2_{s+a} *(Option A):* Ten kernel samples were treated as in option A; individual dilution and plate count series were prepared from each extract.
$\sigma^2_{s+a'}$ *(Option B):* Ten kernel samples were each treated as follows: after grinding the kernels, the subsampling variance was omitted by extracting all of the meal. This was achieved by dividing the meal into ten equal portions, treatment as in option B, followed by pooling of the ten resulting extracts. From this pool, one decimal dilution and plate count series was prepared.

Combined subsampling and analytical variances $\sigma^2_{ss+a'}$:
Ten meal samples were treated as in option B; individual decimal dilution and plate count series were prepared from each extract.

Results and Discussion

Mean values of colony forming units ($\overline{cfu/g}$) and the analytical variances and coefficients of variation (C.V.) of the kernel method (option A) and the meal method (option B) are presented in Tables 1 and 2, respectively. When compared with the meal method, the kernel method results in higher absolute cfu values with a smaller C.V.. A possible explanation is that in option B, mould propagules tend to become entrapped by the suspended meal particles to a varying degree, depending on the particle size distribution of the meal. Uneven grinding of kernels would thus contribute to increased variance in the meal method.

Table 1 Analytical variance of kernel method (Option A)

n	$\overline{cfu/g}$	σ^2_a	C.V. (%)
10	$1.38 \cdot 10^5$	$1.09 \cdot 10^9$	23.93

Table 2 Analytical variance of meal method (Option B)

n	$\overline{cfu/g}$	$\sigma^2_{a'}$	C.V. (%)
10	$4.28 \cdot 10^4$	$3.76 \cdot 10^8$	45.36

Table 3 Sampling variance vs. sample size

sample size (g)	n	$\overline{cfu/g}$	σ^2_{s+a}	$\sigma^2_{s+a'}$	C.V. (%)	σ^{2*}_{s}	C.V. (%)* $_{s}$
50	10	$2.03 \cdot 10^5$	$1.59 \cdot 10^{10}$		62.07	$7.19 \cdot 10^9$	57.27
250	10	$1.58 \cdot 10^5$	$9.10 \cdot 10^9$		60.35	$6.72 \cdot 10^9$	55.40
500	10	$9.31 \cdot 10^4$	$2.41 \cdot 10^9$		52.69	$4.83 \cdot 10^9$	46.94
500	10	$2.52 \cdot 10^4$		$2.72 \cdot 10^8$	65.41	$1.93 \cdot 10^8$	47.13
1000	10	$2.99 \cdot 10^4$		$3.03 \cdot 10^8$	58.27	$1.17 \cdot 10^8$	36.58
2000	10	$3.06 \cdot 10^4$		$2.79 \cdot 10^8$	54.50	$7.89 \cdot 10^7$	30.21

*) Calculated by $\sigma^2_{s+a} = \sigma^2_s + \sigma^2_a$ (Option A) or $\sigma^2_{s+a'} = \sigma^2_s + \sigma^2_{a'}$, (Option B), based on $\overline{\overline{cfu/g}}$ = $1.48 \cdot 10^5$ (Option A), resp. $\overline{\overline{cfu/g}} = 2.94 \cdot 10^4$ (Option B)

Table 4 Subsampling variance vs. subsample size

subsample size (g)	n	$\overline{cfu/g}$	$\sigma^2_{ss+a'}$	C.V. (%)	σ^{2*}_{ss}	C.V. (%)* $_{ss}$
10	10	$1.72 \cdot 10^4$	$2.13 \cdot 10^8$	84.87	$4.45 \cdot 10^8$	71.73
25	10	$2.92 \cdot 10^4$	$4.75 \cdot 10^8$	74.74	$3.06 \cdot 10^8$	59.40
50	10	$3.10 \cdot 10^4$	$3.17 \cdot 10^8$	57.48	$1.08 \cdot 10^8$	35.31

*) calculated by $\sigma^2_{ss+a'} = \sigma^2_{ss} + \sigma^2_{a'}$, based on $\overline{\overline{cfu/g}} = 2.94 \cdot 10^4$ (Option B)

The combined sampling and analytical variances are presented in Table 3. From the combined variances, the sampling variances were estimated for each sample size using $\sigma^2_{s+a} = \sigma^2_s + \sigma^2_a$, based on the cfu/g for each method. Although a significant reduction of σ^2_s can be expected from increased sample size, the effect is less pronounced as was reported by Whitaker [1] for aflatoxin in maize.

The combined subsampling and analytical variances are presented in Table 4. The subsampling variances for each subsample size were obtained in the manner described previously. The influence of subsample size on σ^2_{ss} was found to be more pronounced than in the case of sample size vs. σ^2_s. This might be explained by the reduced particle size of the subsample which makes better mixing possible.

Comparing options A and B it is concluded that, not withstanding the possibility with option B to reduce sampling and subsampling variances considerably by increased sample and subsample sizes, the inherent analytical variance of option B results in a total variance which is higher than that achieved with option A. The variances presented in Tables 1-4 represent single samples, analysed with single analyses (no replications). Under these conditions, option A yielded a C.V. of 52.7 % with 500 g sample size, whereas option B gave a C.V. of 64.2 % with 2,000 g sample size and 50 g subsample size.

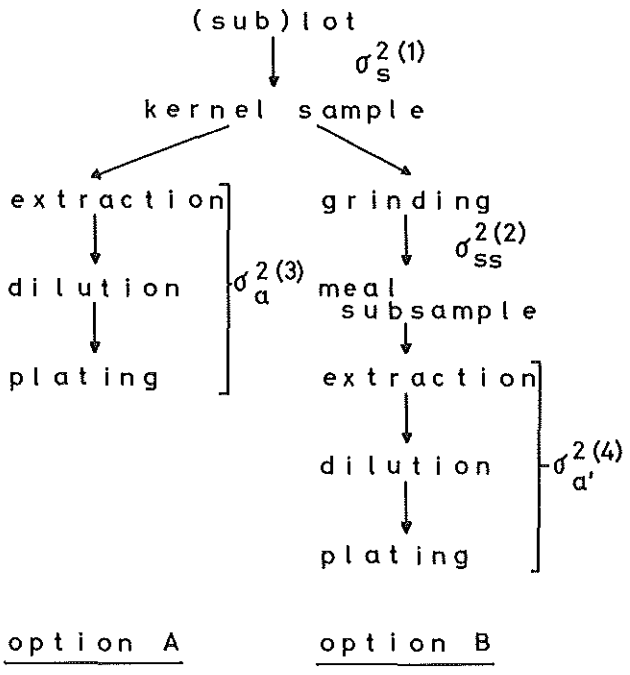

Fig. 1 Optional protocols for the microbiological analysis of maize kernels
(1) sampling variance
(2) subsampling variance
(3) analytical variance kernel method (Option A)
(4) analytical variance meal method (Option B)

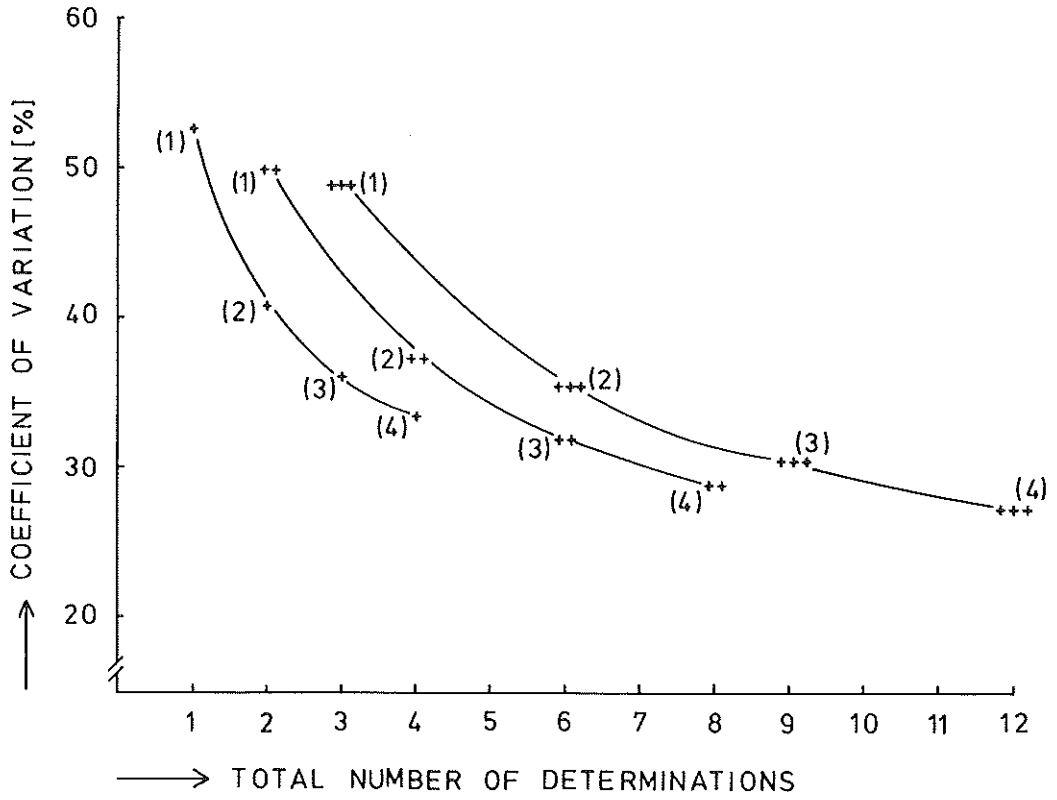

Fig. 2 Influence of replication of samples and analyses on total variance, expressed as coefficient of variation (kernel method, Option A, sample size 500 g)
+ single analysis
+ + duplicate analyses
+ + + triplicate analyses
(..) number of replicate samples

Reduction of the total variance will be achieved by analysing replicate samples or by carrying out replicate analyses from the same sample(s). Figure 2 shows that, due to the fact that the sampling variance is larger than the analytical variance, more benefit is derived from increasing the number of replicate samples, rather than the number of replicate analyses.

Sampling variance estimated from ash content of wheat flour, was favourably reduced by use of an in-line automatic sampling device [3]. Similar improvement might be achieved with the sampling of maize kernels.

It should finally be noted that the variance data presented are valid only for the investigated lot of maize, and that they may not be mapped onto other lots with unknown distribution of mould propagules. Their presentation solely serves to illustrate the order of magnitude of the components of total variance and the extent to which these are affected by protocol and sample size.

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