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Sampling of cereals: assessment of alternative protocols for mycotoxin analysis

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Context and objectives

European directives[1,2] for official controls of some contaminants in cereals such as mycotoxins set methods for sampling and analysis. The sampling protocols are strict and not very practical.

The composition of batches of cereals is rarely homogeneous and, in particular, certain contaminants like Fusarium-mycotoxins are distributed in a non-uniform way. Sampling is therefore a procedure which requires a great deal of care; it is necessary to get a guaranteed representative sample before initiating analysis.

For four years, studies have been undertaken by a French working group of associated storage organizations and suppliers of sampling devices in order to:

- evaluate mycotoxin distributions in cereal batches,
- compare different sampling protocols, including the European directive (reference method),
- determine the relationship between the number of increments and the total analysis uncertainty, and
- define an acceptable sample weight for laboratory analysis.

Evaluation of the distribution of mycotoxins

Three wheat batches of 500T were selected and 100 increments are taken from each. The DON content is determined on each sample by an Elisa test.

Nine maïze batches of 500T to 1500T are selected and 25-150 elementary samples are taken. Fumonisins B1,B2 contents are measured for seven batches, Zearalenone content for three batches and DON content for one batch. Measurements are performed by chromatographic analyses[3,4,5].

The levels of mycotoxins span a wide range of contamination that cover the regulatory thresholds in human food.

For wheat, grids were drawn on the top of the silos in order to ensure a consistent sampling plan across the whole batch. An example of static sampling plan is given Figure 1.

Each of 100 increments are homogenized and ground before being analysed. Mappings are developed based on the results; these all show strong heterogeneity in the silo. This heterogeneity results from the field variability (wheat heterogeneity study conducted by ARVALIS over four years). The silo can be considered as a stack of plots. One level of the silo is made up from different field plots; the observed silo variability is similar to that noted as intra-plot field variability.

The level and the variability of DON contents are very different between silos. The higher the silo's average content, the greater the dispersion.

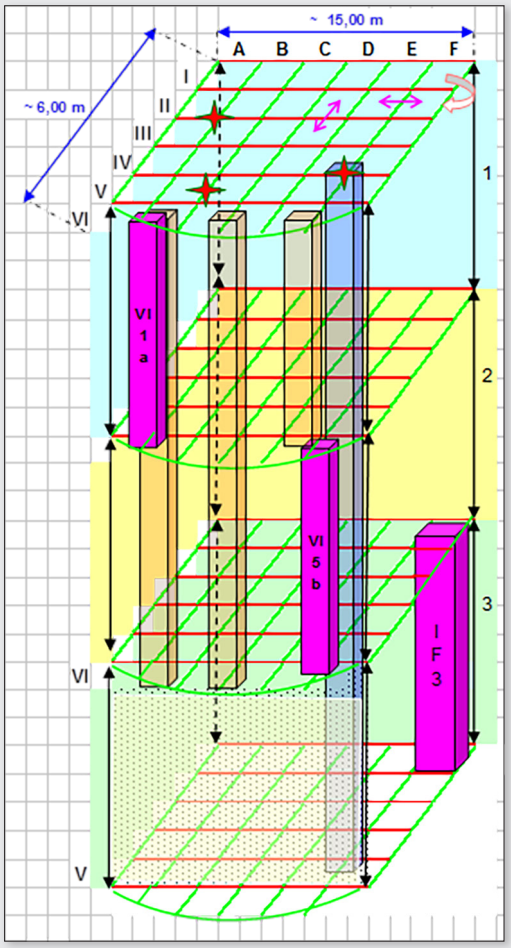

Figure 1. Silo sampling plan.

Table 1. *Mycotoxin* contamination levels

Fusariotoxin	Cereal	Number of silos	Mean silo value (µg/kg)	Food regulatory threshold (µg/kg)
DON	Common wheat	5	477 to 1988	1250
	Maize	1	2633	1750
Fumonisins B1 + B2	Maize	7	534 to 7132	4000
Zearalenone	Maize	3	139 to 683	350

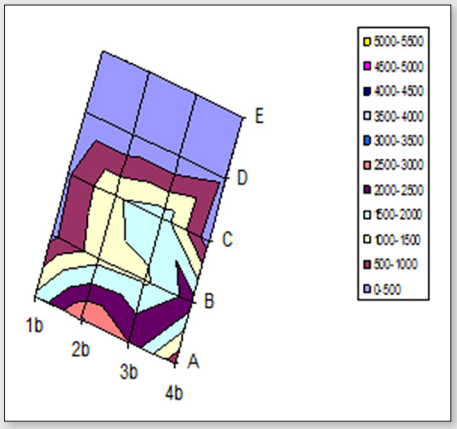

Figure 2. Heterogeneity measured at 4 m depth (DON)

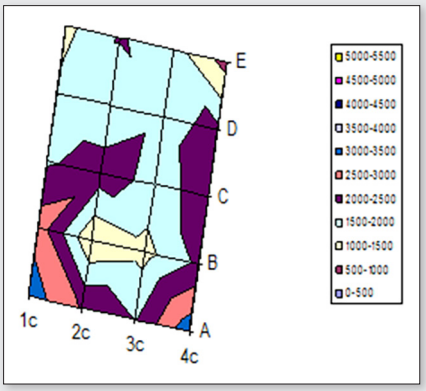

Figure 3. Heterogeneity measured at 6 m depth (DON)

The trials on maize are conducted on flowing grain streams. An increment is taken every 20 to 25 tons. As for wheat, the dispersion of the samples is different depending on the silo. The results recorded for Fumonisins in seven silos show a relationship between average contents and variability.

The higher the mycotoxin contents, the higher the variability between samples. The heterogeneity of batches depends on the level of contamination. The distribution of mycotoxins is not uniform and in addition varies according to the content levels.

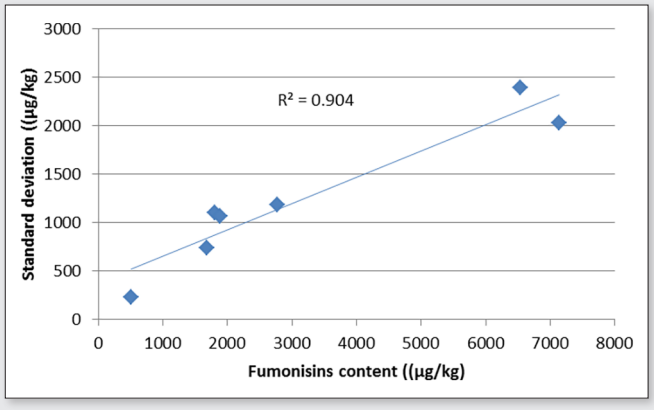

Figure 4. Between-sample standard deviation variability vs. Fumonisin content.

Table 2. Number of increments in alternative sampling protocols

	Reglementary protocol	Normative protocol	Routine protocol
Flowing – 500T	100	25	10
Static – lorry	100	10	3
Static - 500T	100	50	10

Comparison of alternative sampling protocols

The official control of mycotoxins is to be performed on an average sample of the grain batches. Three different protocols for arriving at this average sample are studied here:

- the protocol corresponding to regulation n°401/2006 for fusarium toxins. This is considered as a reference.
- the "normative" protocol drafted by the standardisation working group (EN ISO 24333 standard).This method is adapted to certain situations experienced by cereal operators (e.g. intervention scheme)
- the "routine" protocol with a smaller number of increments than the two alternatives. This protocol is suited to the practical and economic conditions encountered on the daily controls by the cereal industry operators.

Two different grain sampling situations have been taken into account: flowing cereal streams (transfer from one silo to another one, discharge and Redler samplings or train discharge) and static batches (lorries, flat or vertical silos). A total of 22 tests were conducted with a large range of devices. The number of increments for each type of situation is shown in Table 2.

The mycotoxin used for estimation is DON for wheat and maize.

The average DON content of the different batches of grains investigated ranges between 477 and 6,275µg/kg. These values frame the regulatory limits or recommendations well. Statistical analysis of the results (Student's T-test) showed that there is no significant difference between the three protocols. It should be noted however that in the case of lorries, the routine protocol may sometimes misjudge the level of contamination (2 cases out of 14).

A comparison between sampling methods (manual vs automatic sampling) indicated there was no statistically significant difference.

Thus the alternative protocols (normative and routine) *may* be used for estimating the average mycotoxin content of a batch of grain instead of regulatory protocol. A sampling protocol based on a smaller but sufficient number of increments does not lead to an underestimation of the average mycotoxin content of the batch. Use of the regulatory protocol lead to higher, perhaps unjustified costs.

Estimation of the impact of a sampling protocol on accuracy

All test data were used to estimate the error of the estimation of the average mycotoxin content (accuracy).

The global variability observed, characterized by a coefficient of variation (CV), seems to be *independent* of the average content of the silo; it is about 45%.

This variability has two origins: the variability due to sampling errors and that due to the analytical error.

When producing a composite sample obtained from n increments, the sampling variability can be reduced if based on an increased number of individual increments (N.B. covering the full lot volume). By contrast, the analytical error component is constant.

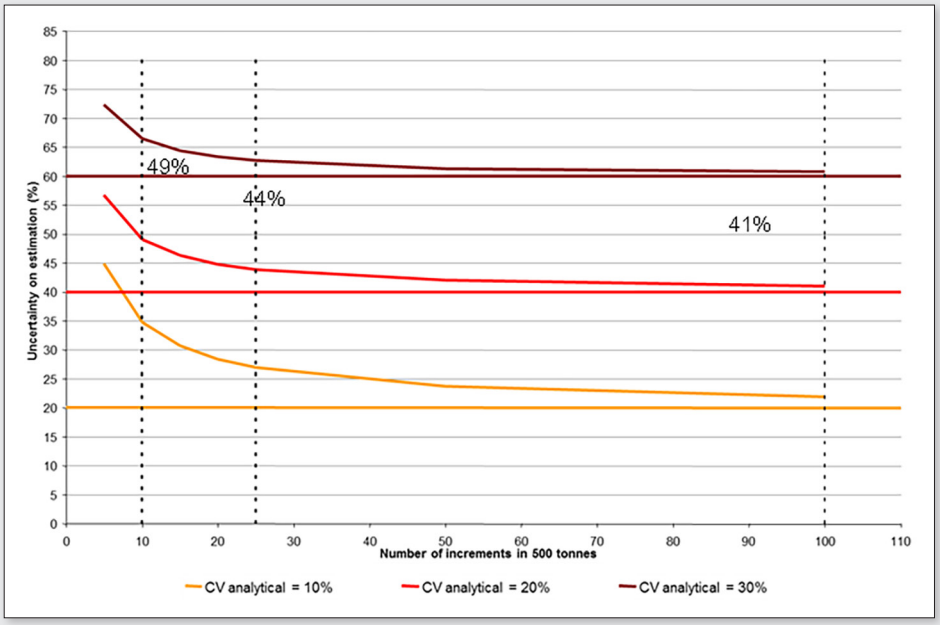

Figure 5. Uncertainty of estimated average mycotoxin content as a function of the number of increments from a 500 T lot.

Statistical simulations were carried out to define the degree of variability due to sampling over that of the chromatographic analysis. For this, three hypothetical levels were considered for the analytical error (CV = 10%, CV = 20% and CV = 30%). The variability due to sampling error can be estimated in relation to the number of individual increments, see Figure 5. Here it can be observed that after a certain number of increments, the reduction of the overall uncertainty of the result generated by an additional increments becomes negligible.

Thus the benefit of a higher sampling intensity is low. By increasing the number of increments from 10 to 100, the total accuracy improves only 8% for an analytical uncertainty equal to 40% (CV analytical = 20%). These models are applicable for wheat and maize contaminated with DON, Zearalenone and Fumonisins.

Influence of the reduction of the size of the laboratory sample

The regulation (EC) defines a total sample mass as the result of aggregation of all the increments taken from the lot or batch, specifying its weight to be 10 kg. But no specification is given for the laboratory sample mass. A mass of 10 kg is too big for the laboratory and causes different problems associated with sub-sampling (division), grinding, storage.

Our trials consisted of reducing a 20 kg sample to ≈500g by using a conical divider, see Figure 6. All the split off fractions were ground and analysed by chromatographic methods. Two wheat samples were characterized for DON while two samples of maize were analysed for for Fumonisins B1, B2.

For this study, the averages obtained at each step of division (sub-sampling) are compared to the average calculated using all available data. This latter corresponds to the initial sample of 20 kg (called “reference”).

Two modes of interpretation of the results were applied:

- comparing the means with the reference, using the critical difference (CD) as defined in the standard NF ISO 5725 – 6 [6];
- Assessing the uncertainty that characterizes the dispersion of values around the reference.

The critical difference was estimated from the standard deviation of repeatability specified in the standard used.

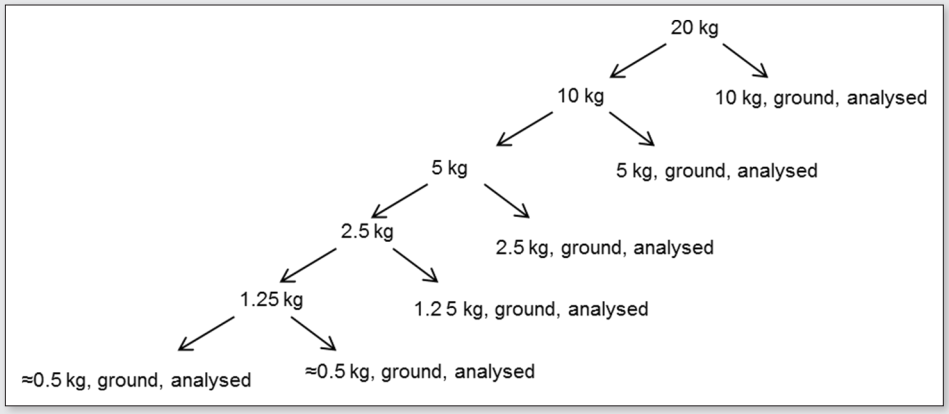

Figure 6. Sub-sampling (division) flow chart.

For DON, only one batch corresponding to a mass of 1kg for one sample showed a greater difference than the DC. For Fumonisins, some differences were observed for fractions less than or equal to 2.5kg.

An uncertainty, Ue, equal to 2 standard deviations of reproducibility from standards was assigned to each reference:

- 39% for DON;
- 34% for Fumonisin B1;
- 39% for Fumonisin B2.

The averages obtained at each step of sub-sampling, for each sample and each level, are included in the intervals of uncertainty associated with references, except for the fraction of 1kg for the 2 maize samples.

The results of this study show that it is possible to suggest the laboratory sample mass as low as 3kg without affecting the estimation of the average level of contamination. It should be noted that it appears possible to reduce this mass to 1kg for the analyte DON.

Conclusions

These studies confirm the significantly high spatial variability of mycotoxin distributions.

They also showed that an average sample composed by a smaller number of increments than that stated in the regulation, may still representative of the target grain lot. The results regarding Fusarium-mycotoxin contents are similar. This means that it is possible to reduce the sampling intensity.

The results were included in the data that supported the drafting EN ISO 24333. This standard, published in 2009[7], has received positive feedback from users. During the review of regulation 401/2006 in 2014[8], the EN ISO 24333 standard has been recognized to sample lots ≥500T and thus reduces the resources devoted to sampling.

The mass of the sample sent to the laboratory for mycotoxin analysis is reduced from 10 to 3kg.

Acknowledgments

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References

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8 Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis.