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Sampling Procedures to Determine the Proportion of Genetically Modified Organisms in Raw Materials

Part II: Sampling from Batches of Grain

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Introduction

The Swiss government introduced a threshold value of 1 % GMO content as the basis of food labelling, 0.5 % for seed and 3 % for fodder. The enforcement of such a threshold requires quantitative detection systems such as quantitative competitive polymerase chain reaction (QC-PCR). Assurance that a sample is representative of the larger lot of material from which it is taken is provided by correct sampling and a sample size large enough to allow analysis to the desired precision.

The sampling process consists of two basic steps:

1. Selection of a primary sample S_1 small enough to be sent to laboratory.
2. Selection of a secondary sample S_2 – a sub-sample of S_1 – small enough to be processed in totality.

In this work it is shown how representative samples can be obtained from batches of grain and how the size of the sample has to be if a limit for an accepted overall error is prescribed. We follow mainly the approach proposed by Pierre Gy who developed a theory of sampling of particulate materials (1). A condensed summary of Pierre Gy's sampling theory and sampling practice can be found in (2). Important elements of the theory are the *constitution heterogeneity* (CH_L), the *distribution heterogeneity* (DH_L), the *fundamental error* (*FE*) and the *concept of correct sampling*. *FE* results directly from the particulate nature of the material sampled. *FE* can never be removed from a sample, but it can be reduced by controlling the maximum particle size allowed into the sample, and increasing the sample size.

It is impossible to prepare a valid plan without first characterising the various kind of heterogeneity carried by GMO's in a particular material. It is essential to

understand where and how the producers are separating their grain throughout the supply chain and where unintentional contamination of genetically modified crops with conventional crops could take place.

Representative field samples (S_1)

Sampling methods to obtain representative field and laboratory samples are given in international standards. Unfortunately many of these standards merely describe practices on which trade has been based for a long time. An example of such a standard is ISO/FDIS 13690 (3) which lays down rules for sampling grain from static bulks or bags. Some of the proposed procedures are only improved grab sampling techniques. For bulk grain exceeding 12 m in depth it is necessary to sample grain when flowing. This latter sampling method is also applicable (and should be applied!) for all depths of bulk grain. ISO 6644 (4) covers sampling of flowing grain. It specifies general conditions relating to automatic sampling by mechanical means and respects the requirements of correct sampling.

Probabilistic sampling of stationary unmovable lots that cannot be mechanically transferred is impossible in most cases. Hand sampling is never probabilistic. The only probabilistic sampling process applicable to unmovable lots of particulate materials is the increment sampling process. Such a process is generally performed during a transfer of the entire lot for a purpose other than sampling. Splitting methods transfer three-dimensional lots into one- or zero-dimensional lots prior to sampling. These lots are usually handling or transportation units such as series of railroad cars, truckloads, shovelfuls, drums, sacks, bags, and so on. If the units are disposed in a natural order reflecting more or less the chronology of their production, the theoretical solution is the one used for one-dimensional lots (2). It supposes that the variogram of the critical content is relatively stable. However, as raw material often coming from different suppliers the variogram cannot be assumed to be stable. In most cases it may not be feasible to proceed with variographic experiments and therefore a zero-dimensional model will be applied.

With units in true random order, the selection scheme is irrelevant. However, there is always a small part of original chronological order that remains; therefore a systematic scheme is recommended. A random stratified scheme would even be safer.

When handling large tonnages under the form of zero-dimensional lots in a routine way, the most accurate and cheapest solution consists of selecting for instance 1 unit (truck, railroad car, container, sacks, bag) out of 10 or 20 (primary sample) according to systematic or random stratified scheme, discharging the increment units in a surge bin, feeding the material to a cross-stream sampler (secondary sample), and feeding back the sampling rejects to the empty barrels kept in standby. The solution requires capital expenditures but leads to very small operating costs and very high reliability, which is very important in commercial sampling.

Representative laboratory samples (S_2)

The technical problems at the secondary sampling stage, i.e. to select a laboratory sample from a field sample, are much easier to handle. For instance, for unprocessed soy beans and maize kernels, analytical samples fully representative of a 2.5 kg field sample can be produced by grinding the whole sample to sufficiently fine powder, mixing thoroughly, and taking two 1 g samples of this powder for analysis. If the opposite sequence were followed, that is if 1 g from the 2.5 kg sample is weighed out and ground to powder hardly a representative sample would result. A 2.5 kg sample of soy, for instance, contains about 10000 individual beans. When ground to fine powder, each bean is reduced to many thousands of fragments. When the powder from all beans in the 2.5 kg sample is mixed thoroughly, each 1 g sample of that mixture contains approximately equal numbers of fragments from every bean in the 2.5 kg sample. On the other hand, if 1 g of the original sample were weighed out for the analytical sample, it would contain only four or five of the 10000 beans in the original sample.

Minimum sample mass of S_1 necessary to reach a given uncertainty

Let L be a lot of N_F constitutive elements of equal mass and true critical content a_L. If a zero-dimensional model is applied and if the sampling selection is correct only a short-range heterogeneity fluctuation error $CE_1 = FE + GE$ is taken into consideration. The fundamental error FE is the relative sampling error committed if a sample S of size n is selected in such a way that each possible combination of that number of units has the same probability of selection (*simple random sampling*). Such a sampling is unbiased (5) with a relative variance of $s^2(FE)$:

$$s^2(FE) = \frac{1}{n} \cdot \frac{N_F - n}{N_F - 1} \cdot \frac{1 - a_L}{a_L} \quad (1)$$

As the constitution heterogeneity CH_L is an estimator of the relative variance $(1-a_L)/a_L$ equation (1) can be approximated by

$$s^2(FE) = \frac{N_F - n}{n \cdot N_F} \cdot CH_L = \left(\frac{1}{M_S} - \frac{1}{M_L}\right) \cdot IH_L \quad (2)$$

where $IH_L = CH_L \cdot \frac{M_L}{N_F}$

is the heterogeneity invariant, M_S the mass of the n elements selected and M_L the total mass of the lot. The order of magnitude of the minimum sample mass M_{S_0} to ensure a given sampling reproducibility can be deduced from equation (2):

$$M_{S_0} = \frac{IH_L}{s_0^2(FE)} \quad (3)$$

If $a_L = 1\,\%$ and $M_{m^*} = 0.3$ g (average mass of a maize kernel) it follows that $IH_L = 0.3 \cdot (1-0.01)/0.01 = 29.7$ g (table 1). Let the maximum acceptable fundamental

error $s_0(FE)$ be 10 %. To reach a given sampling reproducibility (at a 95 % level) of ±20 % a minimum sample mass of M_{S_0} = 2970 g (≈9900 kernels) would be necessary (table 2).

Segregation and grouping error

When collecting increments to make up a sample an additional segregation and grouping error (*GE*), has to be taken into consideration. Its variance can be expressed as $s^2(GE) = \gamma \cdot \xi \cdot s^2(FE)$, where γ is a grouping factor and ξ a segregation factor. The grouping factor is always positive, and characterises the size of the increments making up a sample. The value of the segregation factor is always between 0 and 1 and characterises the amount of segregation. It is important to notice that both factors cannot be dissociated from one another and it is always their product that has to be taken into account. The grouping factor is minimised by taking as many and as small increments as practically possible, assuming as the delimitation, extraction and preparation of these increments are carried out correctly. As a rule of thumb based on numerous experiments a sample should be made up of at least 30 increments (1).

The only method to reduce the segregation factor ξ is to homogenise the whole batch before sampling. However, this is not always possible because of economical reasons. Homogenisers are usually limited to the size of laboratory equipment. Large amounts of variographic experiments (1) showed that the product $\gamma \cdot \xi$ is generally slightly below one. In absence of variographic experiments a pragmatic estimate of the variance $s^2(CE_1)$ of the discontinuous heterogeneity fluctuation will be $s^2(CE_1) = s^2(FE) + s^2(GE) \leq 2\, s^2(FE)$.

It would be further meaningless to choose an allotted sampling variance $s^2(TE)$ considerably smaller than the analytical variance $s^2(AE)$ since the reduction of the overall variance $s^2(OE)$ would be very small and relevant only to the second order. However, a too large sampling variance would ruin the advantage of a precise analysis. Therefore it would seem logical to allow for a sampling variance equal to the analytical variance. However, it is usually cheaper to allow for a total sampling variance $s^2(TE)$ slightly larger than the allotted analytical variance $s^2(AE)$.

Let $s^2(OE)_{max}$ be the maximal accepted variance of the overall relative error *OE*, $s^2(AE)$ the analytical variance and assume $s(FE) \approx s(GE)$. Then the minimum sample mass M_{S_0} to reach a sampling reproducibility of $\pm 2s(OE)_{max}$ is determined by

$$M_{S_0} = \frac{2IH_L}{s^2(OE)_{max} - s^2(AE)} \quad (4)$$

When a given sampling reproducibility of ± 20 % should be reached and if it is assumed that a_L = 0.5 %, M_{m^*} = 0.2 g (average weight of a soy bean), $s(FE) \approx s(GE)$ and $s(AE)$ = 5 %, the minimum sample mass M_{S_0} needed is M_{S_0} = (2·0.2·0.995/0.005)/(0.01–0.0025) = 10613 g (table 3).

Table 1 lists the values of the heterogeneity invariant IH_L in grams for four different commodities: flower, wheat, soy and maize and four different critical contents: 0.1 %, 0.5 %, 1 % and 3 %.

Table 1
Heterogeneity invariant IH_L in grams

		Critical content			
Commodities	M_{m^*} in g	0.1 %	0.5 %	1 %	3 %
Flower	0.00001	0.010	0.002	0.001	0.000
Wheat	0.04	39.96	7.960	3.960	1.293
Soy	0.25	199.80	39.800	19.800	6.467
Maize	0.30	299.70	59.700	29.700	9.700

Table 2 displays the minimum sample weight in grams if grouping and segregation error *GE* and analytical error *AE* are negligible.

Table 2
Minimum sample mass M_{S_0} necessary to reach a sampling reproducibility (95%) of ±20 % if grouping and segregation error *GE* and analytical error *AE* are negligible

		Critical content			
Commodities	M_{m^*} in g	0.1 %	0.5 %	1 %	3 %
Flower	0.00001	1.0	0.2	0.1	0.03
Wheat	0.04	3996	796	396	129
Soy	0.25	19980	3980	1980	647
Maize	0.30	29970	5970	2970	970

Table 3 lists the minimum sample mass in grams if $s(FE) \approx s(GE)$ and $s(AE) = 5\,\%$.

Table 3
Minimum sample mass M_{S_0} in grams necessary to reach an overall estimation error (at a 95 % level) of ±20 %. It is further assumed that $s(GE) \approx s(FE)$ and $s(AE) = 5\,\%$

		Critical content			
Commodities	M_{m^*} in g	0.1 %	0.5 %	1 %	3 %
Flower	0.00001	2.66	0.53	0.26	0.09
Wheat	0.04	10656	2123	1056	345
Soy	0.25	53280	10613	5280	1724
Maize	0.30	79920	15920	7920	2587

Table 4 presents the overall estimation error (in %) if $s(FE) \approx s(GE)$ and $s(AE) = 5\,\%$ and the laboratory uses a 2.5 kg sample of raw material.

Table 4
Overall estimation error (in %) if the laboratory uses a 2.5 kg sample of raw material and if it is assumed that $s(GE) \approx s(FE)$ and $s(AE) = 5\%$

		Critical content			
Commodities	M_{m^*} in g	0.1 %	0.5 %	1 %	3 %
Flower	0.00001	10.0	10.0	10.0	10.0
Wheat	0.04	37.1	18.8	15.1	11.9
Soy	0.25	80.6	37.1	27.1	17.5
Maize	0.30	98.4	44.8	32.4	20.3

Conclusions

- Grab sampling, purposive sampling and sampling with thief probes and auger are non-probabilistic selection processes. Only splitting and cross-sampling from a flowing stream provide representative samples.
- Crucial is the primary sampling stage, i.e. the selection of a field sample representative of the whole lot.
- As a rule of thumb a sample should be made up of at least 30 increments.
- A threshold value of 0.1 % can hardly be enforced. To test such a low limit laboratories have to grind much more than 20 kg of soy beans or maize kernels. This is not practical in routine analysis economically.

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Summary

Pierre Gy's sampling theory and sampling practice is applied to select representative samples and to determine the minimum sample mass necessary to allow analysis to the desired precision. Only splitting and cross-sampling from a flowing stream provide representative samples. The minimum sample mass is much larger than usually expected.

Zusammenfassung

Pierre Gy's Theorie und Praxis der Probenahme wurde angewandt, um repräsentative Stichproben zu gewinnen. Repräsentative Teilproben aus umfangreichen Materialmengen können nur mit einer mechanischen Probenahme mit geeigneten Geräten aus in Bewegung befindlichen Materialmengen gewonnen werden. Probenteiler können nur bei kleinen Materialmengen verwendet werden. Je nach gewünschter Genauigkeit ist eine mehr oder weniger umfangreiche Stichprobe

erforderlich. Der Umfang dieser Stichprobe ist weit grösser, als gemeinhin angenommen wird.

Résumé

Les méthodes d'échantillonnage établies par Pierre Gy permettent la prise d'échantillons représentatifs et de déterminer la taille d'échantillon minimale pour obtenir une précision donnée. Il s'avère que la taille d'échantillon minimale est beaucoup plus grande qu'on suppose généralement. Seul l'échantillonnage par fractions et des coupes transversales de la matière en cours de mouvement sont garants d'échantillons représentatifs.

Key words

Correct sampling, Representative sampling, Sampling uncertainty, Minimum sampling mass, Pierre Gy's sampling theory

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