Sampling in Food Safety and Quality Analysis

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Uncertainty (error) of measurement is the most important single parameter that describes the quality of measurements.
A COMPLETE MEASUREMENT PROCESS

**Process**
- Sampling
  - Physical sample preparation

**Form of Material**
- Sampling target
- Primary Sample
- Subsample
- Lab Sample
- Test sample

**Process Description**
- Sample Collection increment/composite
- Comminution/splitting
- Further comminution
- Physical preparation: drying, sieving/milling/homogenizing
- Selection of test portion for next step
- Chemistry/derivatisation
- Determination of analyte concentration

**Analysis**
Each step contributes to uncertainty (error) of measurement

Measurement uncertainty

Decision uncertainty

Heterogeneity

Sampling uncertainty

Analytical uncertainty
Total Error

Sampling error

Sampling target (Lot)

Primary Sample

Analytical error

Analysis
Sampling is a process for making an inference from analytical data, through multiple mass reduction stages from a decision unit (DU)/sampling target.
### Sampling terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decision Unit:</strong></td>
<td><strong>Material from which sample is collected and inference made</strong></td>
</tr>
<tr>
<td>Increments</td>
<td>Individual portion of material collected by a single operation of a sampling tool and combined with other increments to form a primary sample</td>
</tr>
<tr>
<td>Primary sample</td>
<td>The collection of one or more increments taken from a decision unit according to a sampling protocol</td>
</tr>
<tr>
<td>Laboratory sample</td>
<td>The material received by the laboratory.</td>
</tr>
<tr>
<td>Analytical sample</td>
<td>Prepared from the laboratory sample and from which test portions are removed</td>
</tr>
<tr>
<td>Test portion</td>
<td>The quantity of material taken for measurement.</td>
</tr>
</tbody>
</table>
DECISION UNIT

• The DU defines the material from which the primary sample is collected, clearly delineating the material on which the decision is to be made.
• The choice of decision unit has a large impact on the sampling protocol.
• **The DU sets the scale of decision-making**
• This scale can be based on mass, volume, time, package size, or any other definable criteria.
The entire DU is selected as primary sample. In this case no primary sampling errors are involved. Sampling errors might occur during mass reduction and selection of the test portion, as well as in the analytical stage (Total Analytical Error).
<table>
<thead>
<tr>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Concentration of concern</td>
</tr>
<tr>
<td>Any detectable level. LOD is 1 CFU/100 sq. cm</td>
</tr>
<tr>
<td>Direct inference</td>
</tr>
<tr>
<td>Will be compared to the concentration of concern (LOD).</td>
</tr>
<tr>
<td>DU</td>
</tr>
<tr>
<td>100 sq. cm. There are 10,000 of the DUs.</td>
</tr>
<tr>
<td>Confidence</td>
</tr>
<tr>
<td>99% confident that no more than 5% of the DUs have detectable levels of <em>L. monocytogenes</em></td>
</tr>
</tbody>
</table>

When the entire DU cannot be selected as the primary sample, a representative sample is taken. The primary sampling error, must be minimized to a level for which the desired confidence ensures correct decision making.
A single coffee bean might be defined as the DU. The coffee bean can be individually sampled and analyzed. There is no primary sampling error involved as the entire bean is analyzed (Case 1). Inference can only be made to that particular coffee bean.

Each coffee bag might be defined as the DU. It can either be sampled in its entirety (Case 1), or the coffee bag can be sampled representatively.

Each coffee sack might be defined as the DU. In contrast to the single coffee bean or single coffee bag, the coffee sack cannot be sampled in its entirety (Case 1) in this example due its high mass and can therefore only be sampled representatively (Case 2).
High quality sampling should always be applied to ensure the use of adequate and representative samples as test materials for making the decision.

The laboratory is involved in smaller scale “sampling” processes each time it selects a smaller mass from a larger mass (mass reduction).
Contributors to Global Estimation Error

Decision Unit Heterogeneity
- Distributional heterogeneity
- Compositional heterogeneity

Primary Sampling error
- Tool design and usage
- Accessibility and randomness
- Mass and number of increments
- Analyte integrity and evidentiary

Subsequent Mass reduction error
- Equipment design and usage
- Accessibility and randomness
- Mass and number of increments
- Analyte integrity and evidentiary

Global estimation error
- Primary sampling error
- Mass reduction error
- Analytical error

Adapted from GOODSamples
Errors linked to characteristics of the material under analysis

Sample heterogeneity contributes to the total sampling error in two ways

1. Compositional (Constitutional heterogeneity)

2. Distributional (Spatial and Temporal heterogeneity)
Compositional heterogeneity exists when the individual elements that make up the decision unit exhibit differing concentrations of the analyte of interest.
Reducing compositional heterogeneity

• Mixing and blending does not change compositional (constitutional) heterogeneity.

• The only way to alter the constitutional heterogeneity of any given material is by **comminution** (chopping /crushing / cutting /grinding) or by other methods changing the physical properties of a sample.

• The reduction of the average particle size is the dominating factor in reducing constitutional heterogeneity.
Selecting comminution (grinding) equipment

Consider the following:

- Physical and chemical properties of the material,
- Initial maximum particle size of the material,
- Final desired particle size and the range of permissible particle sizes,
- Needed capacity and throughput,
- Inertness to analyte of interest,
- Complete sample recovery,
- Ease of cleaning, disinfecting, and sterilization.
Distributional (Spatial and Temporal) heterogeneity

- Distributional heterogeneity results from non-random distribution of elements within the decision unit (e.g., settling of small, dense fines to the bottom of a container).
- Gravitational forces on particles of different densities, sizes and shapes which cause grouping and segregation of all particles (Grouping or segregation error)
Mixing and blending are techniques that can reduce the grouping and segregation error. Grind ingredients to a uniform particle size range. Incrementing also helps to reduce the distributional heterogeneity.
Coarse grinding (or pre-grinding)

When a ‘dry laboratory sample’ is composed of lumps or its particle sizes are above 6 mm the whole laboratory sample should be pre-ground (e.g. using a cutting mill, or chopped to particle sizes in the range of 4 to 6 mm) before mass reduction / subsampling.
Subsampling (splitting) process.

- A common laboratory preparation practice is splitting of samples for mass reduction.
- The way in which subsampling is achieved is also a source of possible errors.
- Any subsampling process produces its own sampling errors.
- Subdivided into three main types:
  1. increment delineation errors
  2. increment extraction errors
  3. increment preparation errors.
For three-dimensional samples (material spread in a long pile) it is important to subsample also at the very bottom layer to avoid the increment delineation error.

The extraction is done incorrectly because of the extraction tools (e.g. round-edged shovel)
## Sampling tools

<table>
<thead>
<tr>
<th></th>
<th>Correct Design</th>
<th>Incorrect Design</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spatula</strong></td>
<td><strong>Square edged:</strong> Prevents material from falling off each side</td>
<td><strong>Flat without edges:</strong> material segregates when falling from the sides</td>
</tr>
<tr>
<td><img src="image1" alt="Spatula" /></td>
<td><img src="image2" alt="Spatula" /></td>
<td><img src="image3" alt="Spatula" /></td>
</tr>
<tr>
<td><strong>Scoop</strong></td>
<td><strong>Square shape:</strong> All the material has the same chance to be a part of increment</td>
<td><strong>Round shape:</strong> Material at the top has greater chance to be part of the increment than at the bottom</td>
</tr>
<tr>
<td><img src="image4" alt="Scoop" /></td>
<td><img src="image5" alt="Scoop" /></td>
<td><img src="image6" alt="Scoop" /></td>
</tr>
<tr>
<td><strong>Shovel</strong></td>
<td><strong>Square shape:</strong> All the material has the same chance to be a part of increment</td>
<td><strong>Round shape:</strong> Material at the top has greater chance to be part of the increment than at the bottom</td>
</tr>
<tr>
<td><img src="image7" alt="Shovel" /></td>
<td><img src="image8" alt="Shovel" /></td>
<td><img src="image9" alt="Shovel" /></td>
</tr>
</tbody>
</table>
When a small mass is desired from heterogeneous material in the laboratory sample, comminute the entire laboratory sample to a fine particle size or keep the test portion large.

Use a grinding method that produces particles of uniform size and shape.
Subsampling procedures for mass reduction when only a part of the Laboratory sample is used

Common splitting techniques used

- Rotary splitting,
- Fractional shoveling,
- Stationary riffle splitting
- Coning and quartering

- All these techniques include incrementing.
- When a small number of increments is selected, there can be very large subsampling errors due to sample heterogeneity.
- The number of increments has to be determined in view of the size of the acceptable error and not according to the ease of handling.
- As a rule of thumb it is recommended to take 10 increments when there is no prior knowledge about the sample heterogeneity
Rotary Splitters
The basic components of a riffle splitter include the scoop, an even number of chutes, or riffles, and a pair of collection pans.
Coning and quartering repeatedly divides a sample into halves until the desired sample size is achieved. Not recommended as error is large.
Fractional (alternate) shoveling
It is a very simple splitting technique with the following advantages:
- No costly equipment involved
- Minimal clean up and decontamination requirements;
- Can be implemented in the lab or field
- Any number of splits can be generated
- Very low sample splitting error.
Minimum is three splits.
The laboratory sample is split into the desired number of samples by collecting increments from the laboratory sample. Increments that should be of identical sizes are alternately placed in containers or piles to create split subsamples.
A) The ‘S-shaped’ pattern used for spreading the laboratory sample in a tray B) Random spread location of the extracted increments.

B) The spoon method can be used when a relatively low sampling ratio is needed (reduction by less than a factor 10).
Mass reduction by the long pile method

- The sample is poured and spread on flat surface in a long pile.
- The pile should have a length of at least 20 times its width and the height should not exceed the width of the pile.
- Several random cross sections are then taken out of the pile and all the cross sections put together lead to a mass reduction of the original sample.
Analyte integrity

No change in Physical, chemical, biological and/or radiological characteristics of interest in the decision unit.

- Oxidation/Reduction
- Microbial viability/contamination
- Volatilization
- Degradation (Temperature, light, moisture)
- Adulteration/contamination
- Changes in moisture content
- Temperature (storage)

Considerations for analyte integrity include preservatives, aseptic containers, maintaining proper temperature (refrigeration/freezing) and humidity, protection from UV light.
Quality Control: Levels of replication using triplicates

- Replicates can be implemented at multiple points to sort out error contributions from various mass reduction stages.
Quality Control: Levels of replication using triplicates

Replication of analytical samples to generate multiple test portions provides estimation of error associated with selection of:

1. Analytical sample
2. Test portion
3. Test
Quality Control: Levels of replication using triplicates

- Replicating test portions from the same analytical sample provides an estimate of the imprecision associated with selection of the test portion and the test
Data resulting from test portions can be used to estimate total error with repeatability of the whole process
In summary

• Food materials are routinely analysed for: risk analysis, compliance with regulatory requirements, post-market monitoring, surveillance, and manufacturing process control.

• Analytical results are of low value, no matter the quality of the method used, if the sampling process is imprecise and not representative of the entire field-to- aliquot pathway.

• All sampling processes, irrespective of the nature of their decision units (target lots) need to be structurally correct to make correct decisions.
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Open access publications


Additional Resources

- GOODSamples, 2015
- Report EUR27021EN - Guidelines for sample preparation procedures in GMO analysis TECHNICAL REPORT · JANUARY 2014 DOI: 10.2788/337005
- Ramsey, Charles A. The Decision Unit – A Lot with Objectives. TOS Forum, 5, 2015. Doi: 10.1255/tosf.75
- Ramsey, Charles A. The Role of Inference in Food Safety. TOS Forum, 5, 2015. Doi: 10.1255/tosf.77
Particle size reduction

Comminution, the generic term for particle reduction can be achieved through several processes:

- **Chopping**: a material is mechanically cut into smaller parts;
- **Crushing**: applying pressure to particles to fragment them into smaller particles; especially for samples with large and hard lumps,
- **Cutting**: cutting mills reduce soft to medium-hard and fibrous materials using rotating and stationary cutting knives;
• **Blending** (homogenising): materials that are generally semi-solid (i.e. with a certain water content) are mixed with rotating knives which break fragments in smaller particles and blend the entire sample to a macroscopically more uniform texture (at least in appearance) and consistency (e.g. ‘Waring blenders’);

• **Milling / grinding**: reduces solid particles by a combination of cutting, shearing and pressure;

• **Pulverising**: describes the action of various mills which further reduce small-sized material (< 10 mm) to an average particle size usually below 75 µm.
Formula to calculate the mass increment

\[ m_{\text{(increment)}} = \frac{m_{\text{(lab sample)}}}{n_{\text{(subsamples)}} \times N_{\text{(increments)}}} \]

- \( m \) = mass and \( n \) and \( N \) = number

- All increments must have approximately the same size;
- Each subsample must have an identical number of increments;
- Split subsamples must be randomly selected
- All material must be used.
Maintaining integrity of the laboratory sample

To minimise the loss of integrity of DNA molecules of the sample, heating during the grinding process must be limited (e.g. when grinding some fatty matrices like linseed with a 0.5 mm mesh size sieve).
Mixing techniques

• Mixing is used by many laboratories in an attempt to ‘homogenise’ the sample.
• Once the sample is ‘homogenised’, any increment or grab from the sample is then deemed representative of the sample without any further considerations.
• This assumption does however not hold true for particulate matter.
Unacceptable Mixing Techniques

- Putting a spatula in the top of a sample container and stirring it a few times vertically.
- For dry, ground material, shaking a sample container when the jar is full or almost completely filled.
- The material at the bottom of the container is not properly mixed in and the stirring might have actually promoted segregation thus enlarging the mass reduction error.
- It is recommended to leave at least one third of open space in the jar.
A simple technique: The plastic bag technique
A simple technique: The plastic bag technique

The ground material is put in a plastic bag that can be closed (sealed, zipper). Leave approximately one half of open space in the bag. Homogenization occurs through 20 successive reversals of the bag. Although it may depend on the nature of the material, for commonly treated samples the following bag sizes can be considered:

- for 1 kg of ground sample: a plastic bag of 30 cm by 40 cm,
- for 2 kg of ground sample: a plastic bag of 37 cm by 50 cm.
Samples may need partial drying before grinding

- For some semi-solid matrices with less than 85 % dry matter (e.g. forages, fresh bread, but not for liquids), it is necessary to partially dry them prior to fine grinding.
- Partial drying can be done using either a forced-air oven or a microwave oven.
- The aim is to dry the sample while keeping its temperature below 55–60 °C to minimally affect its chemical composition.
- Drying at higher temperatures (i.e. above 60 °C) might cause chemical changes which could affect the DNA content of the sample.
- Following drying, the subsample is ground.
Special sample preparation procedures

Gelatine, molasses and samples with high fat content
• Cool the entire laboratory sample in a freezer overnight and process the sample in a frozen or chilled state;
• Use dry ice during grinding to keep the sample cold enough to prevent clumping or melting;
• Use blending type mills, operated in intervals of 30 s.

Viscous samples
• For viscous samples such as fluid lecithin and honey heating the sample at 40–60 °C improves the homogeneity of the sample and helps obtaining a representative sub sample
**Liquid samples**

- Liquid samples are generally considered as homogeneous) and do not require prior homogenisation.
- For oil where DNA is present in solid particles that may be in suspension in the liquid. The laboratory sample is submitted to a centrifugation of 30 minutes at ~ 18 000 g. The supernatant is discarded and DNA extraction is performed on the pellet.
Plant tissues

• Fresh tissues: freeze the material in the presence of liquid nitrogen using a mortar and pestle or eventual other disruption devices to crush the material;
• Freeze-drying the material (which might take more than one day) and subsequently grinding the dried material.
Recommended laboratory sample sizes according to the type of matrix

<table>
<thead>
<tr>
<th>Products</th>
<th>Recommended laboratory sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td>Mass equivalent of 3 000 kernels (see table below for mass equivalent of 1 000 kernels)</td>
</tr>
<tr>
<td>Commodity grains</td>
<td>Mass equivalent of 10 000 grains (see table below for mass equivalent of 1 000 kernels)</td>
</tr>
<tr>
<td>First transformation products (semolina, flour, grits, oilcake etc.)</td>
<td>From 100 g to 1 kg</td>
</tr>
<tr>
<td>Liquids</td>
<td>500 ml</td>
</tr>
<tr>
<td>Doughy and viscous products</td>
<td>500 g</td>
</tr>
<tr>
<td>End products (e.g. packed rice noodles)</td>
<td>From 100 g to 1 kg</td>
</tr>
</tbody>
</table>

(Table adapted from AFNOR, 2006)
## Food matrices that don’t need homogenisation or grinding

<table>
<thead>
<tr>
<th>Main Food type</th>
<th>Product that can be considered as homogeneous (which does not mean that comminution is not necessary, it might be required for the DNA extraction step)</th>
<th>Product that cannot be considered as homogeneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal corn and rice products and bakery products</td>
<td>Noodles, dumplings, etc. of corn and rice</td>
<td>Maize and rice flour or mixes</td>
</tr>
<tr>
<td>Edible vegetables and derived products</td>
<td>Bread, cakes, pastry biscuits</td>
<td>Maize kernels, maize for popcorn, sweet maize, rice kernels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corn flakes, muesli, pop corn</td>
</tr>
<tr>
<td>Roots and tubers</td>
<td>Potato starch, potato flour and flakes of potato</td>
<td>Potato, cooked potatoes</td>
</tr>
<tr>
<td>Oil seeds</td>
<td>Soybean flour</td>
<td>Soybean kernels, linseeds, canola seeds, cotton seeds</td>
</tr>
<tr>
<td>Fruit</td>
<td>Papaya juice</td>
<td>Papaya (if coming from one same fruit, it is genetically homogenous — however avoid taking the seeds)</td>
</tr>
<tr>
<td>Vegetable drinks and products derived from vegetable milk</td>
<td>Soy milk and drink, soy cheese, béchamel, soy yoghurt, tofu, soy fluid</td>
<td></td>
</tr>
<tr>
<td>Baby food</td>
<td>Soy milk vegetable baby food</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous edible preparations</td>
<td>Ice creams and sorbets, desserts, puddings, creams, custards, products coming from extrusion processes (tortilla chips), energy bars (for this latter ones, only if detection is addressing genetically modified soybean or maize)</td>
<td></td>
</tr>
<tr>
<td>Meat imitates</td>
<td>Textured soy protein products</td>
<td></td>
</tr>
</tbody>
</table>
## Minimum mass of test portion

<table>
<thead>
<tr>
<th>FSE (expected RSD) →</th>
<th>Maximum particle size ↓</th>
<th>25 %</th>
<th>20 %</th>
<th>10 %</th>
<th>5 %</th>
<th>2 %</th>
<th>1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mm</td>
<td>0.02 g</td>
<td>0.03 g</td>
<td>0.13 g</td>
<td>0.5 g</td>
<td>3 g</td>
<td>12.5 g</td>
</tr>
<tr>
<td></td>
<td>0.75 mm</td>
<td>0.07 g</td>
<td>0.1 g</td>
<td>0.42 g</td>
<td>1.7 g</td>
<td>10.5 g</td>
<td>42 g</td>
</tr>
<tr>
<td></td>
<td>1 mm</td>
<td>0.16 g</td>
<td>0.25 g</td>
<td>1 g</td>
<td>4 g</td>
<td>25 g</td>
<td>100 g</td>
</tr>
<tr>
<td></td>
<td>2 mm</td>
<td>1.28 g</td>
<td>2 g</td>
<td>8 g</td>
<td>32 g</td>
<td>200 g</td>
<td>800 g</td>
</tr>
<tr>
<td></td>
<td>3 mm</td>
<td>4.3 g</td>
<td>6.7 g</td>
<td>27 g</td>
<td>108 g</td>
<td>672 g</td>
<td>2688 g</td>
</tr>
<tr>
<td></td>
<td>4 mm</td>
<td>10.2 g</td>
<td>16 g</td>
<td>64 g</td>
<td>256 g</td>
<td>1600 g</td>
<td>6400 g</td>
</tr>
</tbody>
</table>

NOTE: for materials with densities other than 1 g/cm³, this table can be modified by multiplying the minimum mass with a corrective factor corresponding to the density of the material expressed in g/cm³.

The yellow cells are those compatible with common sample intakes for DNA extraction (maximum 5 g).