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Animal feeding stuffs — Guidelines for sample preparation

Aliments des animaux — Lignes directrices pour la préparation des échantillons

Reference number
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Foreword

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ISO 6498 was prepared by the European Committee for Standardization (CEN) Technical Committee TC 327, *Animal feeding stuffs — Methods of sampling and analysis*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 6498:1998), which has been technically revised.

Animal feeding stuffs — Guidelines for sample preparation

1 Scope

This International Standard specifies guidelines for the preparation of test samples from laboratory samples of animal feeding stuffs, including pet foods.

NOTE 1 The guidelines mostly derive from those developed by AAFCO (see Reference [7]).

The guidelines are overruled by special instructions and regulations for sample preparation demanded by specific analysis methods.

NOTE 2 Such analysis methods are developed by ISO and CEN.

NOTE 3 This International Standard does not include special guidelines for sample preparation for microbiological analysis of microorganisms like yeasts, bacteria and moulds. Nonetheless, for microorganisms which are used as feed additives (probiotics), some important aspects of sample preparation are addressed.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1 Definitions concerning “sample”

2.1.1
lot
quantity of material that is assumed to be of the same production process and represented by specified sampling rules

NOTE For the purposes of this International Standard, the rules are those of Commission Regulation (EC) No. 152/2009.[3]

2.1.2
laboratory sample
sample as prepared (from the lot) for sending to the laboratory and intended for inspection or testing

2.1.3
test sample
subsample or sample prepared from the laboratory sample and from which test portions will be taken

2.1.4
test portion
quantity of material drawn from the test sample (or from the laboratory sample if both are the same)

2.1.5
reserve sample
material left over from the laboratory sample when divided or subsampled test samples have been taken and on which no further particle size reduction is done

NOTE If, for example, mycotoxin or genetically modified organism analyses are done on the whole laboratory sample, then the reserve sample is also reduced to the corresponding particle sizes. The reserve sample should be stored under conditions maintaining integrity.

2.2 Definitions concerning "parameters"

2.2.1
parameter
analyte or constituent or microorganism for which the feeding stuff is to be analysed by microscopic, microbiological, biological or chemical procedures

2.2.1.1
stable parameter
analyte or constituent or microorganism which does not degrade during sample preparation on common handling or storage at room temperatures of 20 °C to 25 °C

2.2.1.2
unstable parameter
analyte or constituent or microorganism which degrades during sample preparation on common handling or storage at room temperatures of 20 °C to 25 °C because they are volatile, degradable, or sensitive to temperature, light, enzymatic degradation or chemical oxidation

NOTE Stability of parameters in this context refers only to the influence of sample preparation, such as intensive grinding, and not to a minimum shelf-life specified by producers or on the label, e.g. for a feed (additive).

Table 1 — Classification (in general) of stable or unstable parameters and reasons for degradation with a view to sample preparation

Origin	Stable parameters	Unstable parameters	Reason(s) for degradation/change
Nutrients	(Crude) protein, fat, ash, fibre	Moisture	Temperature (volatile)
	Starch, sugar, lactose	Ammonia	Temperature (volatile)
	Gas production and enzyme-soluble organic substance production in *in vitro* tests	Organic acids (e.g. lactic acid, acetic acid, butyric acid, fumaric acid, formic acid)	Temperature (volatile)
	Minerals (e.g. Ca, P, Mg, Na, K, Cl)	Unsaturated fatty acids	Air oxidation (can result in production of short-chain fatty acids)
Feed additives	Trace elements (e.g. Cu, Zn, Mn, Fe, Se, Co)	Vitamins (e.g. vitamin A, C, D, E)	Temperature, ultraviolet (UV) light, air oxidation (sensitive)
	Amino acids (e.g. lysine, methionine, tryptophan)	1,2-Propanediol, ethylene glycol	Temperature (volatile)
	Enzymes (e.g. phytases, non-starch polysaccharide enyzmes)	Microorganisms like probiotics (e.g. *Saccharomyces cerevisiae*, *Enterococcus faecium*)	Temperature (freezing), pressure (sensitive to grinding); moisture/dryness (influences growth of microorganisms)
Undesirable substances	Heavy metals (e.g. As, Pb, Cd, Hg)	Mycotoxins (e.g. aflatoxin B_1, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin, zearalenone, ergot alkaloids)	Mould growth and change of mycotoxins possible at room temperature; UV light (sensitive – aflatoxin B_1)
	Dioxins and polychlorinated biphenyls (PCBs) with similar effects to dioxins	Drugs, antibiotics, pesticides	Temperature (sensitive)
		Hydrocyanic acid	Temperature (volatile)
Banned substances	Proteins of animal origin	Banned drugs, banned antibiotics	Temperature (sensitive)
(Other) Microorganisms		Yeasts, bacteria, moulds	Temperature (sensitive), dryness, influx of oxygen (anaerobiosis)

2.3 Examples of animal feeding stuffs characteristics

Some examples of animal feeding stuffs characteristics are given here to assist with the identification and grouping of a laboratory sample based on the terms and annexes used in these guidelines.

NOTE Definitions of animal feeding stuffs are given in legislation worldwide. Sample definitions from European directives and, for straight feeds, in an alphabetical list from a German committee are given in References [4][5][6][8].

2.3.1
birdseed
seeds that are intended to feed birds

EXAMPLES Grains and oilseeds.

2.3.2
whole cottonseed
unprocessed cottonseed product, including the hulls, lint, and meat

2.3.3
mineral mix
supplementary feed that mainly consists of mineral ingredients in either granular, bead or small pellet form and which is free flowing as an entire mix

NOTE Mineral pellets are an agglomerated mineral mix formed by a mechanical process (in general).

2.3.4
dry feeds
feed ingredient or complete animal feed which typically contains a moisture mass fraction of not more than 15 %

NOTE Dry feed pellets are an agglomerated dry feed produced by a mechanical process (in general).

2.3.5
green fodder
edible parts of plants, other than separated grain, that can provide feed for grazing animals or that can be harvested for feeding, including browse, herbage, and mast

NOTE Generally, the term refers to more digestible material in contrast to less-digestible plant material, known as roughage.

2.3.6
silage
forage preserved in a succulent condition by organic acids produced by anaerobic fermentation of sugars in the forage

2.3.7
roughage
fibrous, coarsely textured parts of plants

EXAMPLES Stovers, straws, hulls, cobs, and stalks.

2.3.8
hay
aerial portion of grass especially cut and dried for animal feeding

2.3.9
haylage
forage preserved in a succulent condition by organic acids produced by anaerobic fermentation of sugars in the forage with a moisture mass fraction of about 45 %

2.3.10
total mixed ration
TMR
single mixture of all feed ingredients (forages, grains, and supplements) that is supplied to an animal for a 24 h period

NOTE In practice, the 24 h allotment of the mixture may be offered in one or more feedings.

2.3.11
byproduct
product which remains after processes for the production of ingredients from plant material

EXAMPLE Dried distillers grains with solubles (DDGSs) from fermentation.

2.3.12
oilseed
any seed from which oil is extracted

EXAMPLE Sunflower seeds.

2.3.13
large block feed
molasses block feed
agglomerated feed compressed into a solid mass that is cohesive enough to hold its form

NOTE Large block feed weighs over 1 kg, generally about 20 kg. It may be marketed as a mineral block or a "caramelized" molasses drum, containing various minerals and nutrients. Samples may be received by the laboratory as large chunks, cores or "sticky clumps".

2.3.14
liquid feed
feed product not solid and not aeriform

NOTE A liquid feed contains sufficient moisture to flow readily and may contain molasses.

2.3.15
canned pet food
feed product for pets which has been processed, packaged, sealed and sterilized for preservation in cans or similar containers

2.3.16
semi-moist feed
meat-based feed product for pets or aquatic animals that has been partially dried to prevent microbial decomposition

NOTE The moisture mass fraction may range from 15 % to 40 %. The product is generally in the form of strips or cubes and is designed to be stored at room temperature.

2.3.17
dog chew
rawhide bone
meat and skin or peel strip that has been nearly completely dried to a leather-like consistency

2.3.18
premixture
mixture of one or more micro-ingredients with diluent or carrier

NOTE Premixtures are used to facilitate uniform dispersion of the micro-ingredients (e.g. vitamins, probiotics, drugs or antibiotics) into a final feed.

2.3.19
range and alfalfa hay pellet
agglomerated feed formed by compacting and forcing the mix through, for example, square openings by a mechanical process

NOTE The pellets are mostly about 2 cm in diameter and 5 cm in length (volume about 16 cm^3) and may contain molasses; this definition also applies to alfalfa cubes (chopped alfalfa hay) of larger dimensions.

2.3.20
texturized feed
sticky feed
mix of assorted grains and commercial feed (generally pelleted), all of which has been treated with a coating of, for example, molasses

NOTE Some of the grains may have been steam heated or rolled prior to incorporation into the texturized feed.

2.3.21
aquatic feed
feed which is fed to aquatic animals and which has been mechanically processed into encapsulated pellets, flakes, crumble, and as packaged sealed powder

2.4 Definitions concerning “sample preparation procedure”

2.4.1
homogeneity
degree to which a property or a constituent is uniformly distributed throughout a quantity of material

NOTE Homogeneity may be considered to have been achieved in a practical sense when the sampling error of the processed portion is negligible compared to the total error of the measurement system. Since homogeneity depends on the size of the units under consideration, a mixture of two materials may be inhomogeneous at the molecular or atomic level, but sufficiently homogeneous at the particulate level. However, uniform visual appearance does not ensure compositional homogeneity.

2.4.2
partial drying
part of the sample preparation procedure for feedstuff samples with a high moisture content (dry mass fraction $<$85 %), in which the sample is carefully dried to allow subsequent sample preparation procedures to be applied, such as particle size reduction by grinding with a mill

NOTE 1 The partial drying procedure depends on the feeding stuff [e.g. at temperatures below 55 °C to 60 °C for silages], and on the heat stability of the parameters (e.g. 70 °C $\pm$ 10 °C for drugs and antibiotics).

NOTE 2 Samples for microbiological analysis should not be dried (at temperatures above 40 °C).

NOTE 3 Partial drying can also be achieved by a freeze-drying procedure, which is a careful drying process using a vacuum to allow moisture to evaporate.

2.4.3
coarse grinding
first grinding step of the whole sample when the laboratory sample contains large lumps or when its particle size is above about 6 mm before mass reduction

NOTE Coarse grinding is a special kind of particle size reduction that ensures homogeneity of the laboratory sample for subsampling purposes.

2.4.4
mass reduction
part of the sample preparation procedure to reduce the mass of a laboratory sample by dividing or subsampling it using (stationary or rotary) dividers or fractional (alternate) shovelling, without changing the consistency of the sample

NOTE After mass reduction, all subsamples should have the same properties as the original laboratory sample.

2.4.5
particle size reduction
part of the sample preparation procedure achieved by chopping, crushing, cutting, blending (homogenizing), macerating, milling (grinding), pressing, pulverizing to obtain a homogeneous test sample for further analysis

NOTE In general, particle size reduction follows the mass reduction step of the sample preparation procedure with different sieve size options to ensure integrity of the test sample(s).

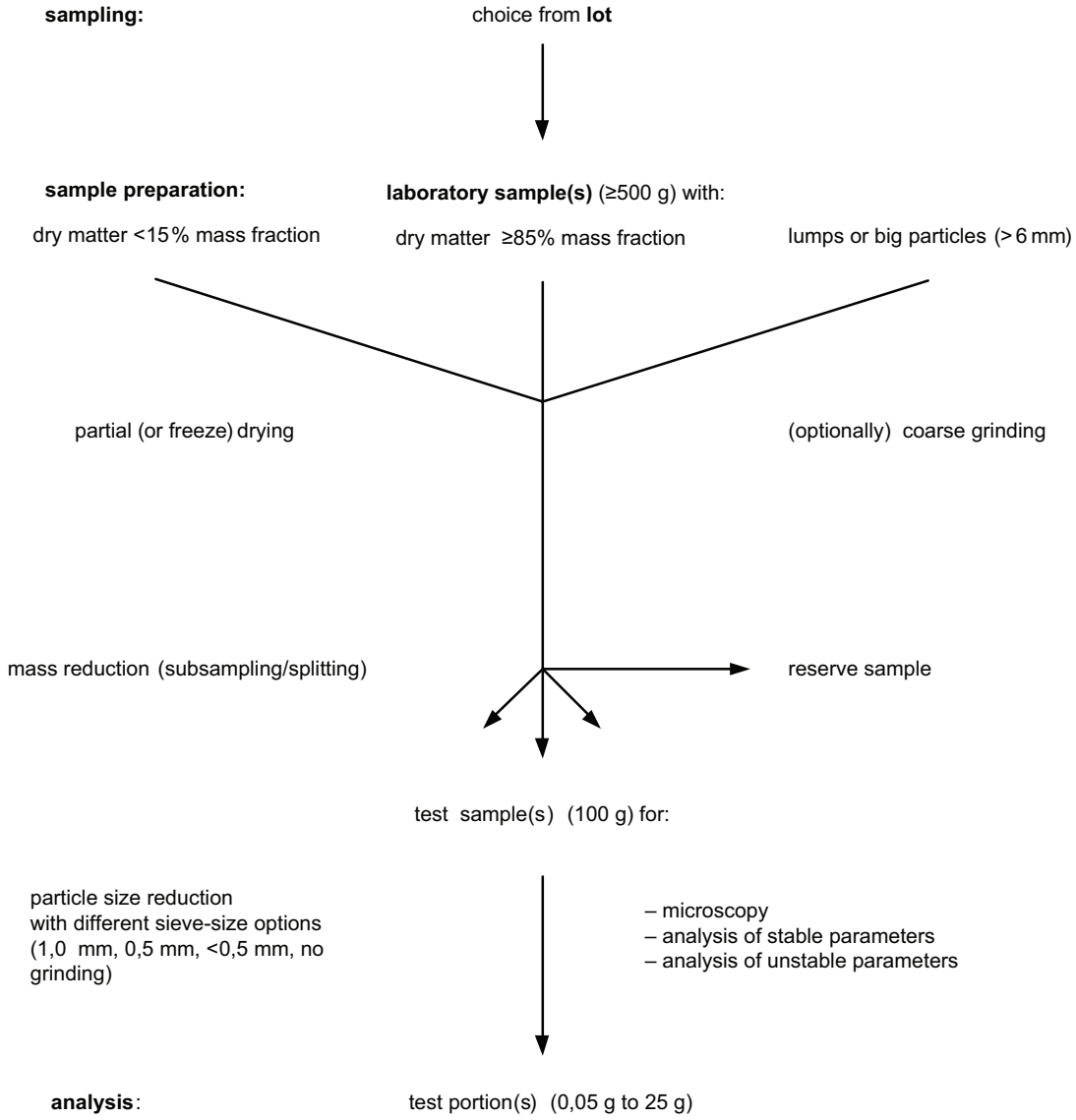


Figure 1 — Illustration of definitions concerning "sample", "substances" and "sample preparation procedure"

3 Principle

All sample preparation steps depend on the different properties of the feedstuffs and on the parameters to be analysed. In each case, any special instructions concerning sample preparation in the analysis methods require consideration.

The guidelines describe the procedure for preparing — from a sample arriving at a laboratory (in general with a minimum mass of 0,5 kg) — a homogeneous test sample (having a minimum mass of 100 g) with the same constitution and composition and free from contamination.

In some cases, the laboratory sample size can be less than 500 g (i.e. in standards for feed additives), but it is necessary to follow statutory regulations and, in every case, the sample size should be large enough to be representative.

In general, the whole laboratory sample is reduced in mass and in particle size to obtain one or more test samples for the analysis of stable and unstable parameters, for microscopy analysis and for reserve. If the analysis protocol and the intended proceeding of the reserve sample permit it, the laboratory sample should preferably be pre-ground completely to an adequate coarse particle size before being reduced further, in order to ensure homogeneity of the test samples.

From a test portion (0,05 g to 25 g and above) prepared for weighing in the feedstuff analysis, representative results should be achieved on the laboratory sample and finally on the whole lot from which the sample was drawn.

Consequently, all steps for sample preparation should be performed quickly, under convenient and very clean conditions, so that there can be no degradation of sensitive analytes, no contamination and no oxidation due to the influence of excessive temperature, daylight, air or residues on the apparatus used or from the samples prepared previously or simultaneously. In particular, contamination from sample to sample should be prevented.

A loss or a change of moisture mass fraction (“content”) during sample preparation should be avoided. In any case, it is necessary to take into account that, in order to be suitable for official control, results require correction (to origin moisture content, dry mass fraction 88 % or 100 %).

For feedstuffs with a higher moisture content (dry matter $<$85 % mass fraction), partial drying or freeze-drying before mass reduction can be necessary.

For feedstuffs with lumps or particle sizes $>$6 mm, coarser grinding of the whole laboratory sample to a particle size of $<$6 mm before mass reduction or subsampling is absolutely necessary.

The samples have to be stored at every stage of the sample preparation under adequate conditions (e.g. at room temperature, refrigerated, frozen, in an airtight container, protected from light or in the dark) to maintain their integrity.

For microbiological analyses, all sample preparation steps need to be done under aseptic conditions. Laboratory samples should be neither frozen nor heated ($>$40 °C), nor subjected to vacuum or oxygen levels higher than those present in atmospheric air.

4 Consideration of sample preparation errors

Sample preparation steps have been shown to be some of the largest sources of laboratory error, a fact which is generally overlooked. This type of error can prove much larger than that arising from subsequent analytical procedures.

4.1 Subsampling and other errors

4.1.1 General

Errors deriving from sample heterogeneity may add to the total subsampling error (TSE) on two levels (Reference [12]).

4.1.2 Constitutional heterogeneity

On a first level, constitutional heterogeneity is a measure of the fact that not all the particles of the laboratory sample have the same composition (shape, size, density, etc.). If a large overall difference between the individual fragments exists, the constitutional heterogeneity is large, but if the fragments are more homogeneous, constitutional heterogeneity is lower. The total contribution to heterogeneity is never zero, however, as that would imply that all fragments are strictly identical. Mixing and blending does not change constitutional heterogeneity. The only ways to alter the constitutional heterogeneity of any given material are by comminution (crushing or cutting) or other methods which alter the physical properties of a sample. The reduction of the average grain size is the dominant factor in reducing constitutional heterogeneity by such means.

Therefore, an initial coarse grinding (pre-grinding) of the whole laboratory sample is necessary before subsampling or division to reduce constitutional heterogeneity.

This fundamental subsampling error (FSE) can be controlled by selecting the test sample mass (see 4.2) appropriately. Therefore, collect enough mass to ensure that particles of all different compositions are contained in the subsample or division. The larger the particle size of a material, the larger the subsample mass has to be to minimize error.

4.1.3 Distributional heterogeneity

On a second level, distributional heterogeneity is a measure of the non-random distribution of particles in the sample, as a result mainly of the action of gravitational force on particles of different densities, sizes and shapes, which leads to a grouping and segregation of all particles. Particles with large differences in size or density tend to segregate or stratify heavily, with the smallest or densest particles sinking to the bottom of the sample. For the sake of illustration, imagine a laboratory sample consisting of black and white spheres and with significantly different grain size distributions. If all the black spheres are found at the bottom of the sample and the white spheres are more to the top, the system displays a very high distributional heterogeneity. If, on the other hand, the spheres were well mixed (homogenized), the distributional heterogeneity of the system would be significantly reduced.

To reduce this grouping and segregation error (GSE), mix or blend the sample before subsampling and collect many increments at random from the laboratory sample (see 4.3).

Mixing is not adequate for many materials. For some materials and circumstances, mixing may actually increase segregation instead of reducing the grouping and segregation error. As long as gravity exists, there will be segregation. Many materials always display an innate propensity for segregation, even immediately after mixing suspensions, e.g. highly density-fractionated materials. Such systems require constant monitoring and treatment but, once this feature has been duly recognized, it can always be dealt with satisfactory.

Incrementing (i.e. the collection of many random increments from the laboratory sample to make up the subsample or division) always works by reducing error from distributional heterogeneity and takes less time and equipment to implement. Thirty increments are generally adequate. More increments are required for very heterogeneous materials and, if little segregation is known to exist, fewer increments can be used, but in no case can fewer than 10 be recommended.

4.1.4 Other errors

Other errors that arise from sample preparation include the loss or gain in analyte content arising from such mechanisms as grinding, excessive heat, loss of fines, contamination, and electrostatic separation. These errors can be large and are usually a result of carelessness or lack of knowledge.

4.2 Minimum mass

To be properly representative of a laboratory sample, the subsample or division shall have adequate mass with a view to fundamental subsampling error (FSE) and maximum particle size (“minimum mass”) (see Table 2).

The mass required depends on the acceptable error in the subsample or division, on the density, heterogeneity, and content of the analyte particles in the matrix, and on the largest particle size (see calculations in Annex A, Examples 1 to 3 and Tables A.1 to A.3).

Table 2 — Minimum mass: expected coefficient of variation (CV) from laboratory subsampling; assumed density, 1 g/cm^3

Maximum particle size mm d	FSE (expected CV) %				
	15	10	5	2	1
	Minimum mass g				
0,5	0,06	0,13	0,5	3	12,5
0,75	0,2	0,4	2	10,5	42
1	0,4	1	4	25	100
2	4	8	32	200	400
5	56	125	500	3 130	12 500

NOTE For materials with densities other than 1 g/cm^3, the entries can be multiplied by the density of the material of interest; for example, the subsampling of a material with a largest particle size of 2 mm, a tolerable subsampling CV of 5 % and a density of 0,5 g/cm^3 would require 16 g.

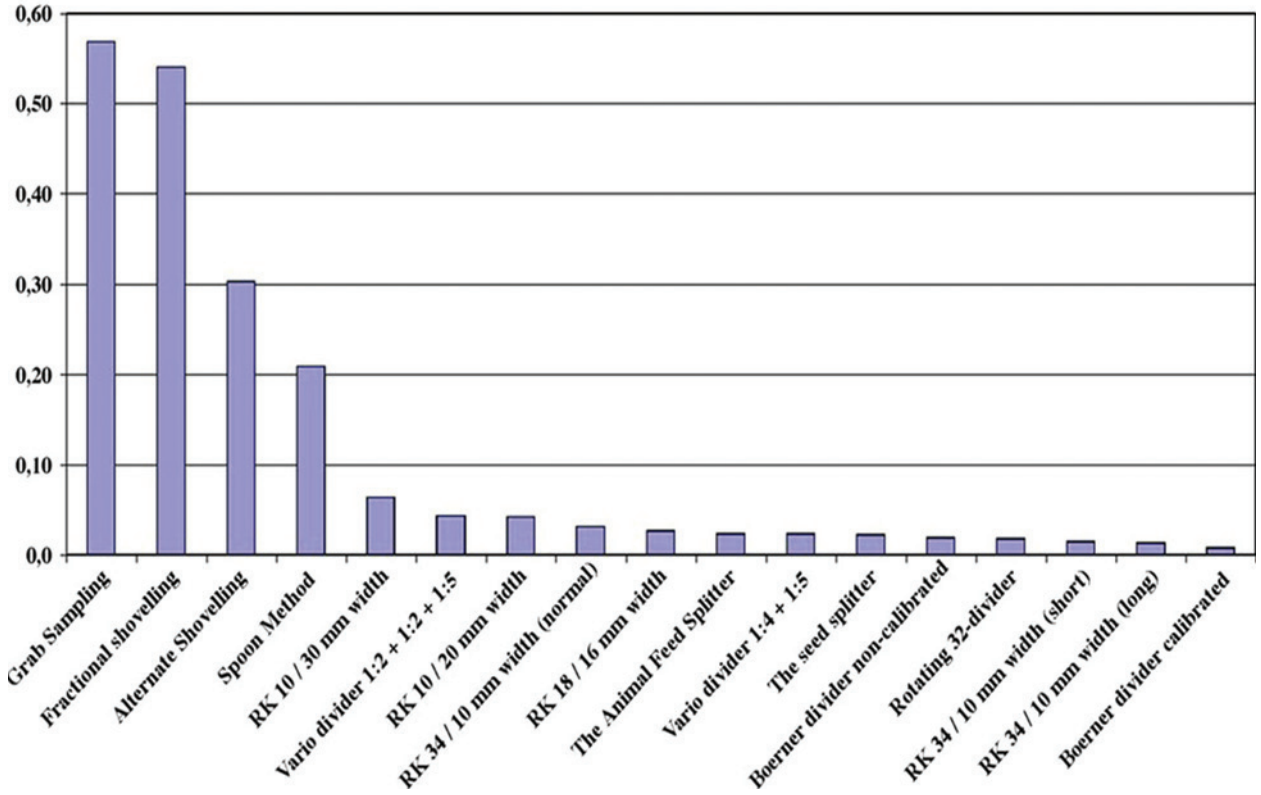
4.3 Errors associated with division techniques

The data in Table 3 demonstrate the error associated with various division techniques for a model mixture of sand particles. Figure 2 demonstrates the representativity (i.e sum of the sampling error related to precision and accuracy) of 17 different mass reduction devices for a model mixture containing mass fractions of 89,9 % wheat, 10,0 % rapeseed, and 0,10 % glass (see References [11][12]). The primary difference in the mass reduction methods is the number of increments selected. For this to be true, structurally correct use of the mass reduction devices is required (e.g. equal probability for the selection of all particles, no loss of particles, centre of gravity rule obeyed, parallel cuts) which is difficult or impossible to obtain with shovelling and grab sampling methods. Therefore mass reduction methods based on grab sampling or shovelling methods can have substantial problems with precision and accuracy on trace components present as separate particles, which may be due to selective loss or poor sampling of smaller particles (see References [11][12]). It can be concluded from Table 6 and Figure 2 that more increments lead to improvements for the mass reduction in the laboratory by reducing the sampling error. In general, a rotational divider reaches several hundred increments, a stationary riffle divider about 10 to 34 increments, and coning and quartering only two increments. Therefore, coning and quartering are not recommended in the critical mass reduction step in the laboratory, i.e. the mass reduction step with the largest contribution to the total error. The preparation of the final test portion, where the ratio between the mass of the laboratory sample and the mass of the final test portion is 100 to 10 000, can usually be considered the critical step of the mass reduction of the laboratory sample. Grab sampling is to be totally avoided for the critical mass reduction step unless it has been established that the sampling error is insignificant compared to the total analytical error.

Table 3 — Test results from division of a mixture containing mass fractions of 60 % coarse sand with 40 % fine sand, P = 0,6 (ISO 664[1])

Method[a]	Number of increments	Standard deviation of samples % s_r	Variance %^{2} s_r^2	Estimated maximum sample error %
Coning and quartering	2	6,81	46,4	22,7
Stationary riffling	10 to 12	1,01	1,02	3,4
Rotary riffling	>100	0,125	0,016	0,42
Random variation		0,076	0,005 8	0,25

[a] Stationary rifflers with a higher number of increments and less subsampling error are available (see Reference [11]).



NOTE Representativity should be as low as possible. Higher sums thus mean lower reliability. RK n indicates a riffle divider with n chutes (see Reference [11]).

Figure 2 — Pooled representativity, r^2, equal to the square of the bias plus the square of the precision, for a model mixture of wheat, rapeseed, and glass

5 Safety precautions

The mills for crushing, cutting and grinding have sharp moving blades. Never put hands or fingers past the edges of the introduction chamber. Never open the mills until they have completely stopped. Check to see that safety interlocks on all equipment are operating properly.

Wear appropriate personnel protective equipment as required in the laboratory. Safety is of great importance during the sample preparation phase of the analysis.

Operate the dust ventilation system during dust generation procedures. To minimize dust, use a vacuum cleaner to clean the hood area, mills, and work area.

Check that all electrical equipment is properly earthed and maintained. Do not place metal items or aluminium foil in the microwave oven when using it for drying samples.

6 Apparatus

Usual laboratory equipment and in particular the following. All equipment used should be appropriate to the risk of contamination and oxidation during sample preparation.

6.1 Equipment for sample preparation in general.

6.1.1 Brushes for cleaning grinders, etc.

6.1.2 Compressed air blower for cleaning.

6.1.3 Vacuum cleaner.

6.1.4 Systems for microbe reduction of mills, **equipment for disinfection** and **flame treatment** for microbiological analysis.

6.2 Drying systems.

6.2.1 Lyophilization system, forced-air drying oven capable of being maintained at 55 °C ± 5 °C or **microwave oven**, household type, or **vacuum oven**.

6.2.2 Moisture dish (pan) made of plastics, aluminium or glass, e.g. with ≥50 mm diameter, ≤40 mm deep.

6.3 Equipment for mass and particle size reduction of "wet" feeds (e.g. forages, silages).

6.3.1 Garden pruning clippers for cutting forages or a **paper cutter** for small sample volumes or a **laboratory forage chopper** for large volumes and a **ceramic cutter**, especially when trace elements are of interest.

6.3.2 Cutting mill with 6 mm and 1 mm screens.

6.3.3 Shearing-type mill with forage head and 1 mm screen.

6.3.4 Riffle sample divider, the minimum chute width shall be at least $2d$ + 5 mm, where d is the diameter of the largest particle.

6.3.5 Sterile cutter or **disinfected mill** when microbiological analysis (e.g. of probiotics) is of interest.

6.4 Equipment for mass and particle size reduction of "dry" feeds (e.g. cereals, mineral mixtures, pelleted feeding stuffs).

6.4.1 Riffle divider.

6.4.2 Rotary divider with vibratory feeder.

6.4.3 Shearing grinding mill equipped with 1,0 mm, 0,5 mm and <0,5 mm sieves.

6.4.4 Cutting mill with 4 mm to 6 mm screens.

6.4.5 Shearing blending mill (e.g. household coffee mill).

6.5 Equipment for the storage of samples.

6.5.1 Sterile bottles with airtight lids (e.g. brown glass bottles for unstable parameters like vitamins) and especially for microbiological purposes.

6.5.2 Wide-mouth bottles with screw cap, plastic.

6.5.3 Plastic bags with low microbe content, with an airtight closure or for setting to vacuum for microbiological purposes.

6.5.4 Refrigerator.

6.5.5 Freezer.

7 Procedure

7.1 General

After registration and a check, including temperature, of a laboratory sample (see 7.2), the homogenization procedure consists of a mass reduction step (see 7.3).

In the second step, the particles in the test samples are reduced to adequate sizes to minimize the subsampling error that arises when the test portion is taken from the test sample. Particle size reduction should be performed without deteriorating the integrity of the substance to be analysed (see 7.4).

For feedstuffs with higher moisture content (dry mass fraction $<$85 %), partial drying below 55 °C to 60 °C can be necessary before grinding a subsample in a mill, to a particle size of 1,0 mm, in order to analyse its stable analytes (see 7.5).

For feedstuffs containing lumps or consisting of particle sizes $>$6 mm, grinding or chopping to particle sizes below 4 mm $\pm$ 2 mm (4 mm to 6 mm) can be necessary before subsampling is possible (see 7.6).

For some fatty or sticky feedstuffs (e.g. oilseeds, pet foods, molasses block feed), special sample preparation procedures are useful or necessary (see 7.7).

Finally, the samples are stored (see 7.8).

Samples taken for routine analysis by near-infrared reflectance (NIR) spectrometry should reflect the sample preparation carried out to derive the calibration. By its very nature, NIR requires minimal or no sample preparation and is often used to analyse samples that are either fresh or have been dried and only coarsely chopped. However, when carrying out a calibration, it should be recognized that, because spectra may be collected and averaged over large samples, it may be necessary to dry, finely grind, and then reduce the mass using a divider to obtain a subsample suitable for reference analysis. Although the spectra represent an average of a larger sample than used to obtain the reference value, this is acceptable practice.

7.2 Sample check

7.2.1 General

First, register the laboratory sample and identify it uniquely (e.g. with a code number).

Before starting the proper sample preparation procedure, certain laboratory sample checks are required.

7.2.2 Check of sample constitution

When arriving at the laboratory, the sample should have sustained no damage and should still be cooled or frozen if necessary (temperature check). Furthermore, the sample protocol should be appropriate to the sample received and all information concerning the sample should be available and complete. Deficiencies (e.g. no information about the type of feeding stuff, open-laboratory sample container, sample protocol not appropriate for the sample container) should be documented and subsequently reported to the principal. If possible, the deficiency shall be corrected. When this is not possible, and the observed deficiency might affect the analytical result (e.g. when there is not enough sample mass, when mould is already present in the laboratory sample due to too high a moisture content or because the sample was not sufficiently cooled during the transport to the laboratory), another sample from the same lot is necessary.

7.2.3 Check of feeding stuff properties

The laboratory sample should be identified for grouping based on the definitions and categories of feeding stuffs (see 2.3).

The moisture content of a laboratory sample determines the initial steps of sample preparation. “Wet” samples with high moisture content (dry mass fraction <85 %) should be prepared as soon as possible or stored at low temperatures, otherwise deterioration occurs.

For forages with moisture contents too high for direct grinding (dry mass fraction <85 %), the whole laboratory sample should be chopped into pieces of about 1 cm. If necessary, the laboratory sample is subsampled by alternate shovelling and subsequently partially dried. The above is recommended for stable analytes and for the whole, or at least remaining, sample for mycotoxin analysis. For unstable (volatile) analytes [e.g. organic acids, ammonia, hydrocyanic acid, as well as for genetically modified organisms (GMOs) and organic residues] just as for microbiological analysis, it is recommended that a test sample be analysed as such, without previous drying of the sample. Alternatively, vacuum drying at low temperatures or freeze-drying could be performed when samples are to be analysed for non-volatile components.

For “dry” feeds composed of lumps or with particles larger than 6 mm, first a coarser grinding of the whole sample (e.g. with a jaw crusher) to particle sizes of 4 mm to 6 mm is recommended before mass reduction or subsampling is initiated.

7.2.4 Check of substances to be analysed

The number of test samples depends on the number of analytes to be analysed.

For the analysis of stable substances and unstable analytes, and for microscopy analysis and microbiological analysis, separate test samples should be prepared. Any laboratory sample remaining is used as reserve (backup).

In the case of stable analytes, this enables the test sample to be reduced immediately to adequate particle sizes and subsequently stored at room temperature until further analysis.Test samples for unstable analytes should be stored at low temperatures and, in order to prevent degradation, they should only be reduced to adequate particle size on the day of analysis (and not too long in advance).

For the testing of the composition of feeding stuffs by microscopy and for (microbiological) analysis (e.g. probiotics), it is important that no particle size reduction by grinding (milling) be performed. Test samples for the analysis of probiotics should not be frozen, only refrigerated (4 °C to 10 °C).

For mycotoxins and analysis of GMOs by polymerase chain reaction (PCR), if possible, the whole laboratory sample, or at least the greater part of the remaining laboratory sample, should be used for particle size reduction and then subsampled if necessary.

After mass reduction, in general, test samples for stable and unstable analytes of the particle size classes listed in Tables 4 and 5 should be prepared under adequate (temperature) conditions: see Table 7 and Table A.1.

Table 4 — Recommended particle sizes of test sample(s) for stable analytes and for microscopy

1,0 mm	Nutrients (e.g. crude protein, crude fat, crude ash, crude fibre, sugar, lactose), if not ground with a sieve size of 0,5 mm; and minerals, trace elements, heavy metals, if not ground with a sieve size of 0,5 mm or 0,1 mm
0,5 mm	Starch, amino acids, methionine hydroxy analogue (MHA)
0,1 mm	In mineral mixes for minerals, trace elements and heavy metals
No grinding	Microscopy analysis (e.g. composition) or NIR/NIT (near-infrared transmittance) analysis or nuclear-magnetic-resonance oil analysis or phytase activity (if not ground with a sieve size of 1 mm)
Cutting to pieces of 1 cm, followed by a reduction to 0,5 mm or 1 mm	In forages for analysis of the corresponding test sample
NOTE Treatment and storage of samples at room temperatures is possible.	

Table 5 — Recommended particle sizes of test sample(s) for unstable (degradable, volatile, heat-sensitive, microbiological) analytes

1,0 mm	In dry feed for moisture, vitamins, organic acids, 1,2-propanediol, organic residues like PCBs, OCDs, other pesticides, antibiotics, veterinary drugs and mycotoxins
0,5 mm	In dry feed for mycotoxins because of non-uniform distribution within a (laboratory/test) sample if not ground with a sieve size of 1,0 mm
Coarse grinding	For cereals and pressed feeds which do not break down sufficiently in suspension solutions for microbiological analysis
No grinding, but soft treating by solving under light pressure	In dry feed, pelleted or as meal, for microbiological analysis (e.g. of probiotics)
No grinding	In mineral mixes and premixtures for vitamins, antibiotics, drugs, and probiotics, when the particle size is not sufficient for vitamins, antibiotics, drugs (not for probiotics), grind briefly to 1,0 mm to avoid heat generation
No grinding, but cutting to pieces of 1 cm	In forages, for analysis of the corresponding test sample with origin moisture content of organic acids, ammonia, hydrocyanic acid, carotene, bacteria, yeasts and moulds
No grinding, but macerating with a thermo mixer	In forages for organic residues like pesticides, antibiotics, veterinary drugs
Particle size reduction of the test samples should be done quickly and on the day of analysis if possible. Heat generation during grinding procedure should be avoided. When the analysis does not start immediately after the preparation of the test sample or test portion, the latter should be stored at low temperatures in a refrigerator. With the exception of test samples to be analysed for microbiological analysis, e.g. of probiotics, it is recommended that test samples or test portions be stored in a freezer when analysis is not started within 48 h of particle size reduction.	

Reserve samples are stored without particle size reduction, but if mycotoxin or GMO analysis by PCR is necessary, the whole laboratory sample should be prepared to the corresponding particle sizes.

Treatment and storage of the reserve sample should be done under conditions which maintain its integrity over an adequate time period (e.g. until the guaranteed minimum shelf-life of a sample is exceeded).

7.3 Mass reduction

Laboratory samples can be mass-reduced by division devices or subsampling.

Mass reduction using rotational dividers or riffle dividers is recommended and these techniques can be used to reduce a 100 g test sample to test portions $<$1,0 g without incurring serious problems.

If it has been established that the mass reduction error is insignificant or if it is not possible to mass-reduce with the correct mass reduction devices (i.e. rotary or riffle dividers), the mass reduction can be accomplished with subsampling. With subsampling, anywhere from a single increment to as many as several hundred increments are selected at random from the primary sample to form the subsample.

Unfortunately, it is common practice to take only a few increments. If only a small number of increments are selected, there can be very large subsampling errors due to sample heterogeneity. The number of increments should not be determined in terms of what is easy, but rather in terms of what is acceptable from the point of view of acceptable error. Rotary riffle division is the most accurate division method. Coning and quartering is a very poor method and should never be used.

If it is known that the material is not segregated, then fewer than 10 increments can be selected. If the material is known or suspected to be heavily segregated, then more than 10 increments should be selected.

Many materials have a wide range of particle sizes when they arrive at the laboratory and need more increments to be properly representative (consider more than 10 increments).

During the sample preparation by grinding and sieving, the range of particle sizes is reduced and fewer increments can be taken.

7.3.1 Mass reduction devices

7.3.1.1 Riffle dividers

Criteria for the design:

— even number of chutes;

— greater number of chutes is desirable;

— for non-gated rifflers, the feeding scoop should be exactly the same width as all the riffles;

— minimum chute width should be at least $2d$ + 5 mm, where d is the diameter of the largest particle — ensure that chutes do not clog with particles, which can occur if the chute width is not adequate;

— rifflers shall be made from durable, inert materials (e.g. stainless steel);

— rifflers with bent chutes or any defects shall never be used.

Criteria for proper use:

— the riffler should be on a firm, level surface;

— do not feed the sample too fast (the chutes can fill up and overflow);

— for non-gated rifflers, do not feed the sample into the riffle divider with the receiving pan (it is not the correct width); material in the feeding scoop should be spread out evenly prior to pouring into the divider; material shall be fed slowly in the centre of the chutes (to prevent overflow of material from the shallow chutes into the deeper ones);

— for gated rifflers, slowly feed the material into the hopper in a back and forth motion; the material should be evenly distributed in the hopper after feeding;

— fine powders should be fed with care, as they can clog the chutes;

— fines may stick to the divider due to static electricity; if this occurs and measurement of the fines is important, earth the riffler or use an antistatic mat.

Riffle dividers are highly operator-dependent. Carry out tests with material similar to the material to be divided to demonstrate their performance.

7.3.1.2 Rotational dividers

Criteria for the design:

— should be made of inert material;

— cutting edge should be radial from centre (pie-shaped);

— maintain constant speed;

— minimize drop from feeding chute to cutting edge to prevent dust formation;

— fine powders should be fed with care as they can clog the holes.

Criteria for proper use:

— use a vibratory feeder to feed the material into the rotary divider; hand feeding results in uneven feeding rates and therefore non-uniform incremental division;

— adjust the feeder rate so that the material flows through the feeder at a continuously slow, even rate without overflowing into the rotary divider; each division (bottle) should contain around 200 increments per division [the recommended minimum of increments per division (bottle) is 50] as a slower feeding rate results in more increments per division (bottle) and therefore in a more representative subsample;

— after division, each bottle should contain an equal volume of material (if the volumes are not equal, this indicates that one or more of the dividing chutes became clogged); when the volumes are not equal, then all the material needs to be recombined and redivided.

If the material contains large particles, it may have to be coarsely ground through a 4 mm to 6 mm screen before division (see 7.6).

7.3.2 Fractional (alternate) shovelling

This is a very simple division technique with the following advantages:

— can be implemented in the laboratory or field;

— does not involve extra equipment (e.g. rifflers);

— has minimal clean-up and decontamination requirements;

— any number of divisions can be generated;

— has very low sample division error.

The laboratory sample is divided into the desired number of samples by collecting increments. The increments from the laboratory sample are alternately placed into containers or piles to form the divided subsamples.

If a sample is to be divided into two equal subsamples, one division would contain the odd increments and the other subsample would contain the even increments.

If a sample is to be divided into three subsamples: the first division would contain increments 1, 4, 7, ...; the second division would contain increments 2, 5, 8, ...; and the third division would contain increments 3, 6, 9, ...

For larger numbers of divided samples, the same pattern shall be followed.

To calculate the mass increment, m_{inc}, use Equation (1):

$$m_{\text{inc}} = \frac{m_{\text{LS}}}{n_{\text{div}} n_{\text{inc}}} \tag{1}$$

where

m_{LS} is the mass of the laboratory sample;

n_{div} is the number of divisions;

n_{inc} is the number of increments.

The following precautions shall be considered:

— all increments shall be of approximately the same size;

— each division shall have the same number of increments;

— divided samples shall be selected at random;

— each division should have at least 30 increments, where possible;

— all the material shall be used.

Towards the end of the division process, there can be a small amount of fines. It is advisable to reduce the increment size so that the fines are equally apportioned between the divisions, with at least 10 increments each. If this is not done, it is possible that all fines incorrectly end up in only one of the divisions.

7.4 Particle size reduction

7.4.1 General methods

General methods include:

— chopping: a material is mechanically cut into smaller parts;

— crushing: applying pressure to fragment larger particles into smaller fragments; in particular, variable-jaw crushers reduce large, hard samples of particle diameter 1 mm to 15 mm;

— cutting: cutting mills reduce soft to medium-hard and fibrous materials using rotating and stationary cutting knives; the size to which it is reduced depends on the sieves used in combination with mill;

— blending (homogenizing): materials are broken into smaller parts and blended to make them more uniform in texture and consistency;

— macerating: a soft material is torn, chopped or cut into smaller pieces;

— milling or grinding: grinding of materials to mechanically reduce particle size is accomplished by cutting, shearing, impacting and attrition using various mills;

— pressing: liquids from semi-solid materials such as plants, fruit and meats are squeezed out for additional analysis;

— pulverizing: describes the action of various mills that further reduces small feed size material (<10 mm) to a final fineness usually below 75 µm.

7.4.2 Requirements for choosing size reduction equipment

Requirements for suitable size reduction methods differ widely and depend on the sample material.

The equipment should not corrupt the subsequent results of analysis (e.g. cause contamination with trace elements or heavy metals like chrome or nickel from abrasion). Identical results shall be achievable in the same lengths of time when the same grinding tools are used.

Considerations for selecting size reduction equipment for a specific application include the following.

— Type of material. How hard is the material? What are the physical and chemical properties? Is the size reduction process affected by heat generation, moisture change or chemical reactions?

— The initial maximum particle size (e.g. chunks, powder, ...etc.).

— The final desired particle size (in millimetres or micrometres) and the range of permissible particle sizes.

— Quantity of material to be ground and number of laboratory samples to be processed daily or weekly.

— Amount of time available for size reduction in the overall sample processing.

— Abrasion resistance of the grinding tools. Contamination due to wear of the grinding or cutting elements by the grinding tools is a constant threat and shall be absolutely avoided. It is important to select suitable grinding elements that are constructed from materials that do not interfere with the analysis. Typically, particle size reduction tools are made of stainless steel, tungsten carbide, agate, sintered alumina, hard porcelain, and zirconium. A tool with harder surfaces than the laboratory sample material is desirable and minimizes contamination.

— Versatility of grinding equipment. Due to the nature of some sample materials, wet grinding may be necessary or the sample shall have to be cooled or embrittled during size reduction. Some materials require grinding in an inert atmosphere, with liquid nitrogen or in a vacuum.

— Requirements for operator time and cleaning equipment. It is impossible to grind laboratory samples without losing minute amounts of sample, because some adheres to the grinding surface. This material is lost during cleaning.

7.4.3 Types of particle size reduction equipment

7.4.3.1 General

There is no industry standard for categorizing particle size reduction equipment. In Reference [7], AAFCO attempts to describe and group equipment currently on the market which is potentially useful for feed laboratories.

7.4.3.2 Crushers

Reduce particle size by crushing the material. Crushers are generally used to reduce a very large particle size (diameters as large as 150 mm) to between 0,5 mm and 1 mm fragments. Other types of mills can then be used to further reduce particle size.

Jaw crushers provide a first step in a sequential reduction of coarse materials. They operate by compressing material in a chamber between two strong breaking jaws — one stationary jaw and one moving jaw. The jaws are located between thick panels that form a duct, which tapers down toward the adjustable discharge gap.

NOTE Modern mills (7.4.3.3) are also capable of reducing very large particles, so a special crusher is no longer strictly necessary.

7.4.3.3 Mills

7.4.3.3.1 General

Mills can be grouped into cutting mills, grinding mills, combined cutting and grinding mills, impact mills, and air jet mills.

7.4.3.3.2 Cutting (shearing) mills

These mills utilize blades or rotors to shear or cut the material and can be classified according to whether material is reduced by rotating blades causing a reduction against fixed blades or by rotating blades throwing the material against a sieve or an abrasive grinding ring. These mills can be purchased as either a floor model with feed sizes of typically 60 mm to 100 mm whose fineness of grind ranges from 0,25 mm to 20 mm depending on the material, or a bench model whose fineness is determined by sieves.

7.4.3.3.3 Grinding mills

Grinding mills can be grouped according to their grinding action (impact, friction, shearing, attrition, etc.) and the corresponding initial and final particle size of the material processed. These include ball mills, centrifugal mills, disk mills, planetary mills, pulverizers, mortar mills, and vibrating cup or ring and puck mills.

— Ball mills have hard balls inside an enclosed grinding jar or bowl that pulverize by impact and mix soft-hard, brittle, and fibrous materials. Material is placed in a bowl (or jar) with grinding balls and rotated. Throughput and milling efficiency can be affected by the size and shape of grinding jars, the rotation speeds, and the number, mass and size of balls added to the grinding jar. Material particle size can generally be reduced from between 5 mm and10 mm to less than 10 µm. Ball mills can perform wet or dry grinding.

— Centrifugal mills, have two types, centrifugal ball mills and centrifugal shearing mills. The “ball” in “ball mills” refers to the rotation of the grinding vessel. In shearing mills, a rotor rotates.

— Disk or burr mills pulverize soft to medium-hard and fibrous material in either a continuous or a batch mode. The material is pulverized by feeding between stationary and slowly rotating grinding disks with radial teeth. Material is gravity-fed into the centre of the stationary disk and is progressively ground more finely as it moves with the sloped grinding teeth until discharged at the outer edge of the disks. Final particle size is set by adjustment of a gap setting. Generally, these mills reduce materials from about 20 mm down to about 0,1 mm.

— Impact mills, e.g. a hammermill. These mills have a fast-moving part which collides with a stationary part, compressing and fracturing material. Rotary hammers include the swing hammer which is designated to break up relatively large pieces. In these mills, further reduction occurs by subsequent impact with casing or screen.

— Planetary mills develop high grinding energy via planetary actions. This type of mill employs a two-way planetary action to grind rapidly by both impact and friction, resulting in a very narrow particle size range. Material to be reduced is placed in a bowl (or jar) with grinding balls and placed on a rotating platform. In planetary action, bowls rotate in a direction opposite to that of the bowl platform and centrifugal forces alternately add or subtract. Grinding balls roll halfway around the bowl, then are projected at high speed across the bowl on to the opposite wall. Grinding is intensified by the interaction of the balls. High-energy planetary action gives a narrow particle size range in shorter grinding times than conventional ball mills with gravity tumbling. This type of mill can be used for dry or wet grinding of soft to hard and brittle materials or for mixing, homogenizing, and emulsifying suspensions and pastes. Generally, particle sizes can be reduced from 10 mm to less than 1 µm.

— Pulverizers are grinding mills that reduce material with an initial particle size of 4 mm to 6 mm down to about 75 µm to 250 µm. There is no common mechanism or mode of action, the only common feature of these mills is the very fine end product.

— Mortar grinders or mortar mills. The mortar mill is an automated version of the traditional pestle and mortar. A graded pestle is connected to a variable speed overhead motor, and the material is crushed by pressure and friction between the grinding bowl and the grinding arm (or pestle). Mortar mills can be used for both wet and dry grinding. The longer the grinding period, the smaller the final particle size. Particle size can generally be reduced from about 8 mm to between 10 µm and 50 µm.

— Vibrating cup mills or ring and puck mills utilize high friction and impact energy to reduce samples. Inside a grinding vessel, a disc or disc-and-ring set are vibrated and accelerated by centrifugal force. These mills are for extremely fast high-energy dry or wet grinding.

7.4.3.3.4 Combined grinding and cutting (cross-beater) mills

These mills utilize both cutting (shearing) and grinding actions to reduce the particle size of a material. Materials are fed through a chamber where samples are reduced repeatedly until they are small enough to fall through a sieve.

7.4.3.3.5 Air jet mills

A high-speed stream of air is created in the grinding chamber by introducing air through nozzles. The material is fed into the steam at controlled rates with a feeder and pulled into the grinding chamber where it undergoes a series of high-velocity collisions resulting in pulverization of the particles. As the stream enters the classifier, properly sized product is trapped by the exciting flow and conveyed to the collector. Oversized particles remain in the stream until sufficiently reduced. Regulation of particle size is achieved by controlling the velocity gradient. The air supply can be a source of clean, compressed air or inert, compressed bottled gas, such as nitrogen. Jet mills are used for materials that are abrasive, sensitive to contamination, sensitive to heat or volatile. Since the sample itself is the grinding medium, sample purity can remain very high. Final particle size is in the 0,5 mm to 45 µm range.

7.4.4 Maintaining the integrity of the laboratory sample

Avoidance of moisture loss and preservation of the integrity of materials that contain thermally labile or volatile components requires that heating during the grinding process be minimized.

Dry ice can sometimes be added directly to a mortar or ball mill to keep samples cool during grinding (dry ice should be prepared from CO_2 that is free from impurities that could contaminate the sample).

Some mills can be fitted with a cooling block to permit the circulation of cool liquid during grinding.

Pulverizing the material under liquid nitrogen can be performed in a cryogenic mill, if lower temperatures are necessary to solidify a material.

When using cooling agents, it is necessary to avoid condensation of moisture on the material to preserve the integrity of the sample.

7.4.5 Mixing techniques

Mixing is used by many in an attempt to homogenize the sample. Once the sample is homogenized any increment or grab from the sample is then deemed representative of the sample without further consideration. Nothing could be further from the truth for particulate matter.

While, in some cases, mixing may be possible, in other cases, it actually promotes segregation. This is especially true for material with divergent particle sizes and densities.

Always use caution when mixing particulate matter. While mixing may be advisable for many minerals, the errors previously discussed still exist and need to be addressed.

One type of mixing technique that is not recommended is the use of a spatula in the top of a sample container to stir vertically a few times.

Shaking a sample container when the jar is full or almost full is another technique that is unacceptable for use on dry, ground material. The material at the bottom is not adequately mixed and the stirring may have actually promoted segregation making the mass reduction error even greater.

If mass reduction is performed with proper alternate shovelling or rotary riffling, then mixing is not an issue and does not have to be performed.

There are many other mixing techniques available that are superior to stirring (e.g. drum mixer, stirring vane mixer, V-shaped double cylinder mixer).

Mixing is a very effective technique to improve mass reduction accuracy for liquids and semi-solid materials (e.g. use a high-shear mixer or an emulsifier for canned pet food and for liquid feeds prior to taking a test portion).

7.5 Partial drying

It is necessary to partially dry “wet” feeds with a mass fraction of less than 85 % dry matter (e.g. forages, total mixed rations, non-liquid foods) prior to fine grinding in order to analyse their stable substances; for unstable substances, partial drying is not possible.

Partial drying can be performed using either a forced-air oven or a microwave oven. The aim is to dry the feeding stuff while keeping sample temperature below 55 °C to 60 °C so that chemical composition is minimally affected. Drying at temperatures greater than 60 °C causes chemical changes in the feeding stuff (e.g. protein degradation). The dried feeding stuff should be equilibrated at room temperature for about 15 min before measuring partial dry matter so as to minimize the potential change in moisture that can occur during grinding and storage. Drying at temperatures lower than 60 °C does not remove all of the water from the feeding stuff; therefore, (initial) partial drying does not represent the total dry matter of the feeding stuff. Following drying, the subsample is ground and analysed for (final) laboratory dry matter (the remaining 3 % to 15 % moisture) when other chemical constituents are determined.

Therefore, a two-step procedure for determining dry matter is recommended. First, determine the partial dry matter content (if less than 85 % dry matter), then determine the remaining dry matter content on a ground test sample and multiply partial dry matter (PDM) by the remaining dry matter (RDM) to determine the total dry matter (TDM) content.

For the analysis of mycotoxins and GMOs by PCR, if possible, dry the entire chopped laboratory sample at a temperature of between 55 °C and 60 °C and grind to adequate particle sizes.

EXAMPLE Procedure for partial drying of forages.

Chop an entire laboratory sample into pieces of 1 cm using hand clippers, a paper cutter or the laboratory forage chopper. Include any ears attached to corn plants. Silages do not need chopping.

— Partial drying using a draft oven.

The entire (chopped) laboratory sample or a subsample of 300 g ± 1 g to 500 g ± 1 g is weighed in a tared vessel (e.g. an aluminium box of dimensions 20 cm × 12 cm × 4 cm) and dried at 55 °C to 60 °C for up to 24 h until the moisture mass fraction is about 8 % to 12 %.

— Partial drying using a microwave oven.

The entire (chopped) laboratory sample or a subsample is weighed in a tared dry paper boat and dried. Drying times and power setting vary depending upon forage type and moisture content.

NOTE "Hot spots" can occur during drying using a microwave oven. A fire can start in a hot spot even though the rest of the forage is still wet. A high fire risk is indicated while mixing the forages between drying cycles if the forage is too hot to touch, if it is smoking or begins to smell charred.

After drying, the box or paper boat is cooled down to equilibrate to room temperature and then weighed to determine the partial moisture content of the partial dried sample using Equation (2):

$$w_{\mathrm{PDM}} = \frac{m_3 - m_1}{m_2 - m_1} \times 100 \tag{2}$$

where

w_{PDM} is the PDM content, expressed as a percentage mass fraction;

m_1 is the mass, in grams, of the container;

m_2 is the inital (wet) mass, in grams, of forage and container before partial drying;

m_3 is the dry mass, in grams, of forage and container after partial drying.

Together with the remaining moisture or RDM content, w_{RDM}, of the partially dried and ground test sample, the TDM content, w_{TDM}, is calculated in order to report the results of the stable analytes determined from the partially dried and ground test sample to either inital (total) dry mass content (equals "fresh mass", FM) of the laboratory sample (as fed to animals or as received in the laboratory) or to 100 % dry mass content.

Equations used to calculate (stable) analytes, A, in arbitrary units, determined from partially dried and ground test sample, A_{PDM}, with PDM content, w_{PDM}, to 100 % dry mass content, A_{DM}, and to initial total dry matter content are:

$$w_{\mathrm{TDM}} = \frac{w_{\mathrm{PDM}} w_{\mathrm{RDM}}}{100} \tag{3}$$

$$A_{\mathrm{DM}} = \frac{A_{\mathrm{PDM}}}{w_{\mathrm{RDM}}} \times 100 \tag{4}$$

$$A_{\mathrm{FM}} = \frac{A_{\mathrm{PDM}} w_{\mathrm{PDM}}}{100} \tag{5}$$

where

w_{TDM} is the TDM content, expressed as a percentage mass fraction;

w_{PDM} is the PDM content of the partial dried sample, expressed as a percentage mass fraction;

w_{RDM} is the RDM content of the pre-dried and ground sample after a further drying step using temperatures of 103 °C ± 5 °C, expressed as a percentage mass fraction;

A_{PDM} is the analyte content determined from the partially dried and ground test sample, expressed in arbitrary units;

A_{DM} is the analyte content calculated to 100 % dry matter, expressed in arbitrary units;

A_{FM} is the analyte content calculated to inital (total) dry mass content (equals “fresh mass”, FM) of the laboratory sample (as fed to animals or as received in the laboratory), expressed in arbitrary units.

7.6 Coarse grinding

When a “dry feed” is composed of lumps or its particle size is more than 6 mm, the whole laboratory sample should be ground by a jaw crusher or a cutting mill or chopped to particle sizes of 4 mm to 6 mm before mass reduction or subsampling is initiated.

Of course, a coarse grinding of the whole laboratory sample to an adequate uniform low(er) particle size before mass reduction is initiated is recommended to ensure homogeneity. However, this is a very time-consuming procedure and aspects for unstable substances require consideration (e.g. heat generation, grinding on the day of analysis), otherwise deterioration starts.

7.7 Special sample preparation procedures

For samples consisting of high fat, gelatin or molasses, some special sample preparation procedures help to get a representative subsample and finely ground test samples:

— place the entire laboratory sample in a freezer overnight and prepare the sample in a frozen or chilled state;

— use dry ice during division and grinding to keep the sample cold enough to prevent clumping or melting;

— use blending-type mills and blend in intervals of 30 s;

— coarsely grind the whole laboratory sample first so that it passes through a 6 mm sieve.

All these steps enable the preparation of fatty or sticky feeding stuffs while subsampling or fine grinding to get representative test samples.

7.8 Storage

Once a representative test sample has been prepared from the laboratory sample, it is essential to maintain its integrity throughout the period it remains in the laboratory, including all analytical processes, reporting of data, and the ultimate disposal of any remaining material.

Proper storage may include storage at reduced temperature (refrigeration or freezing), protection from moisture gain or loss, protection from UV light, etc. to avoid the harmful effect of too many microorganisms which can break down organic compounds.

The appropriate storage conditions vary according to the type of feed material or substance to be analysed.

In deciding upon the proper storage conditions for each material and analyte combination, laboratories need to consider the effect that composition, matrix interactions and chemical or enzymatic activity have on the analyte(s).

Storage and disposal policy should be established and documented within the laboratory to address these issues.

8 Performance tests (quality control)

8.1 General

Performance tests are described to estimate the sample preparation error, which varies according to with the procedure used, the material, the analytes and the analyst.

Performance tests can be used to evaluate new equipment by comparing results from previous equipment.

The principle is to test each preparation step using two or more very dissimilar materials that can easily be separated so that the amount of error can be easily measured.

When choosing materials, maximize the content range of the analyte of interest in the materials. For example, a mixture of sugar and salt can vary between 100 % sugar + 0 % salt and vice versa. A mixture of 9 % mass fraction protein corn and 14 % mass fraction protein cattle pellets has a far less detectable protein error caused by segregation due to its limited maximum protein range. Feed containing low levels of vitamin A supplied in 0,5 mm beads of 650 000 IU/g presents a far greater problem with regard to preparation than simple grain mixtures. Consequently, preparation methods adequate for one analyte (e.g. protein) may be inadequate for another analyte (e.g. vitamin A).

Room temperature, humidity, and air exchange filtration may affect the quality of the sample preparation procedure, especially in climates that suffer major changes in humidity or temperature.

Use the equipment normally used for routine samples. It is essential that performance tests not be restricted to the newest screens or best mills as these give better results that are not so representative of the equipment's performance. The objective is to ensure that all equipment and techniques used by analysts are within requirements.

As an illustration, some performance test procedures are given in 8.2 to 8.4. Adopt these procedures to meet laboratory needs.

8.2 Performance test for mass reduction (division)

The test is performed to evaluate the divider using a heterogeneous mixture. An illustrative example follows.

Use cleaned maize retained on a sieve with a particle size of 5 mm and weigh 400,0 g in a jar. Then add 40,0 g of oats retained on a sieve with a particle size of 4 mm, while discarding the material retained on the sieve with a particle size of 5 mm and passing through the sieve with a particle size of 4 mm. Then add 4,0 g of cleaned alfalfa seed, passing through a sieve with a particle size 4 mm. Mix briefly by tumbling.

Divide using the normal procedure. Separate the components of each division using sieves with particle sizes of 4 mm and of 5 mm and weigh.

Repeat the procedure in the second and third paragraphs for a total of five times.

Calculate the mean and standard deviation of the total division in the right and left divisions. Determine whether the divider gives a 50:50 division.

Calculate the mean and standard deviation of the recovery of corn, oats and alfalfa for the right and left divisions separately. Determine whether there is a bias between the right and left divisions. Calculate the standard deviation.

Maintaining the ratio of components is critical during division. Calculate the percentage recovery in each division for each component as a percentage of the theoretical recovery.

The left maize recovery, $w_{\mathrm{L,m,rec}}$, expressed as a percentage mass fraction, is given by:

$$w_{\mathrm{L,m,rec}} = \frac{m_{\mathrm{L,m,ac}}}{m_{\mathrm{L,m,ac}} + m_{\mathrm{L,o,ac}} + m_{\mathrm{L,a,ac}}} \times 100 = \frac{200}{200 + 20 + 2} \times 100$$

where

$m_{\mathrm{L,m,ac}}$ is the actual left maize mass, in grams;

$m_{\mathrm{L,o,ac}}$ is the actual left oats mass, in grams;

$m_{\mathrm{L,a,ac}}$ is the actual left alfalfa mass, in grams.

Accumulate sufficient data to determine an acceptable variation for each division device.

For rotational dividers, number the jars and compare all No. 1 positions, and so forth.

Table 6 — Example of weighing data (left and right) divisions of three fractions of a mix of maize (400 g, size >5 mm) with oats (40 g, size 4 mm to 5 mm) and with alfalfa (4 g, size <4 mm) after sieving using 4 mm and 5 mm sieve sizes

	Results of weighing after sieving the divisions, g					
	Division 1 (left) Maize >5 mm	**Division 2 (right) Maize** >5 mm	**Division 1 (left) Oats** 4 mm to 5 mm	**Division 2 (right) Oats** 4 mm to 5 mm	**Division 1 (left) Alfalfa** <4 mm	**Division 2 (right) Alfalfa** <4 mm
Replicate 1	200	200	40	40	2	2
Replicate 2	196	204	38	42	1,9	2,1
Replicate 3	190	210	36	44	1,5	2,5
Replicate 4	199	199	39	39	1,9	1,9
Replicate 5	180	200	35	40	1,5	1,7
Mean, g	193	203	38	41	1,8	2,0
s_r[a], g	7	4	2	2	0,2	0,2
$C_{V,r}$[b], %	3	2	5	4	11	12
Ratio 50:50	48	51	47	51	44	51

[a] Standard deviation of repeatability after five replicates.

[b] Coefficient of variation of repeatability after five replicates.

8.3 Performance test for particle size reduction (grinding)

8.3.1 Grind quality and recovery

Clean the mill thoroughly. Record the empty masses of a 20 mesh sieve, 30 mesh sieve, 40 mesh sieve (see Table 7) and the pan. Weigh 200,00 g of shelled maize. Grind and weigh recovered ground material. Sieve using a 0 mesh sieve, 30 mesh sieve, and 40 mesh sieve. Calculate the mass retained on each sieve (total sieve mass minus tare sieve mass) and the percentages on the 20 mesh sieve, 30 mesh sieve, and 40 mesh sieve, as well as the percentage passing through. Calculate percentage recovery by dividing the total mass of material recovered by the initial mass.

Clean the mill and repeat using 100 g of a large granular form of sodium chloride, such as rock salt. Save ground salt for the carryover test.

Percentage recovery, particle size, and carryover vary with different matrices.

Some materials may not grind properly without special treatment (e.g. feeds containing large amounts of molasses are better frozen before grinding). In some mixtures, too fine a grind may result in excessive heating and destruction of analytes such as vitamin A. A mill is definitely too hot when water, fat or urea condense on the neck of the inlet.

Table 7 — Conversion of mesh sieve values into particle sizes

Mesh	Particle size mm	Mesh	Particle size mm
2,5	8,0	50	0,30
3	6,73	60	0,25
5	4,0	80	0,18
8	2,38	100	0,149
10	2,0	140	0,105
14	1,41	170	0,088
18	1,0	200	0,074
20	0,84	270	0,053
30	0,59	325	0,044
40	0,42	400	0,037

8.3.2 Carryover

Prepare a solution of 5 g/l iodine in an aqueous solution of 50 g/l potassium iodide. Store in a dark or actinic bottle and check activity before use by adding a few drops to starch or meal. A deep blue colour appears when the solution is still active.

Detect the carryover cornstarch in the ground salt from the experiment in 8.3.1 by observing the purple-black colour obtained with the iodine-iodide solution. Weigh 10 g of salt into a test tube, add 20 ml of water and mix, then add two drops of starch-iodine reagent. Compare the intensity of colour with known spikes of the ground maize in pure salt. Carryover with routine clean should generally not exceed 50 mg of maize in 10 g of salt or 0,5 g carryover from the 200 g ground.

It is important that the tests reinforce the need to process samples with a procedure which minimizes carryover, such as processing medicated premixes and concentrates separately after processing low-level materials of the same analyte.

8.4 Performance test for mixing

Grinding is often a source of segregation, since harder-to-grind particles may not pass the sieve as quickly as softer particles. Improper mixing may only accentuate this problem.

The mixing container should never be more than two-thirds full, preferably one-half full.

Using a normal storage container, add sufficient ground wheat or similar light-coloured material to fill a quarter of the container volume. Layer on top a quarter volume of a ground-coloured mineral mix. Mix using a rolling motion with the centre of the axis at 45° to the bottle. Record the time needed to mix the contents until they are visually blended. This unmixed-layers method can be used to test any mixing method in the feed laboratory.

Many mixing techniques can become problematic when the sample volume is over half that of the total container. Serious mixing problems generally occur if the container is over three-quarters full.

9 Categories of feeds — Special remarks and flow charts

9.1 General

This clause gives examples of most animal feeding stuffs and their characteristics with some important remarks on the categories listed and corresponding flowcharts to illustrate sample preparation. See Figure 3.

In general, keep all equipment clean to avoid contaminating one sample with another, especially when handling drug and antibiotic samples and particularly when handling materials that have a vitamin A content of more than

1×10^6 IU/kg or have a drug mass fraction in grams per kilogram or milligrams per kilogram. When necessary, wash equipment between samples. Take particular care with premixtures and pure or concentrated additives.

For microbiological analysis (e.g. of probiotics), do not freeze the test sample.

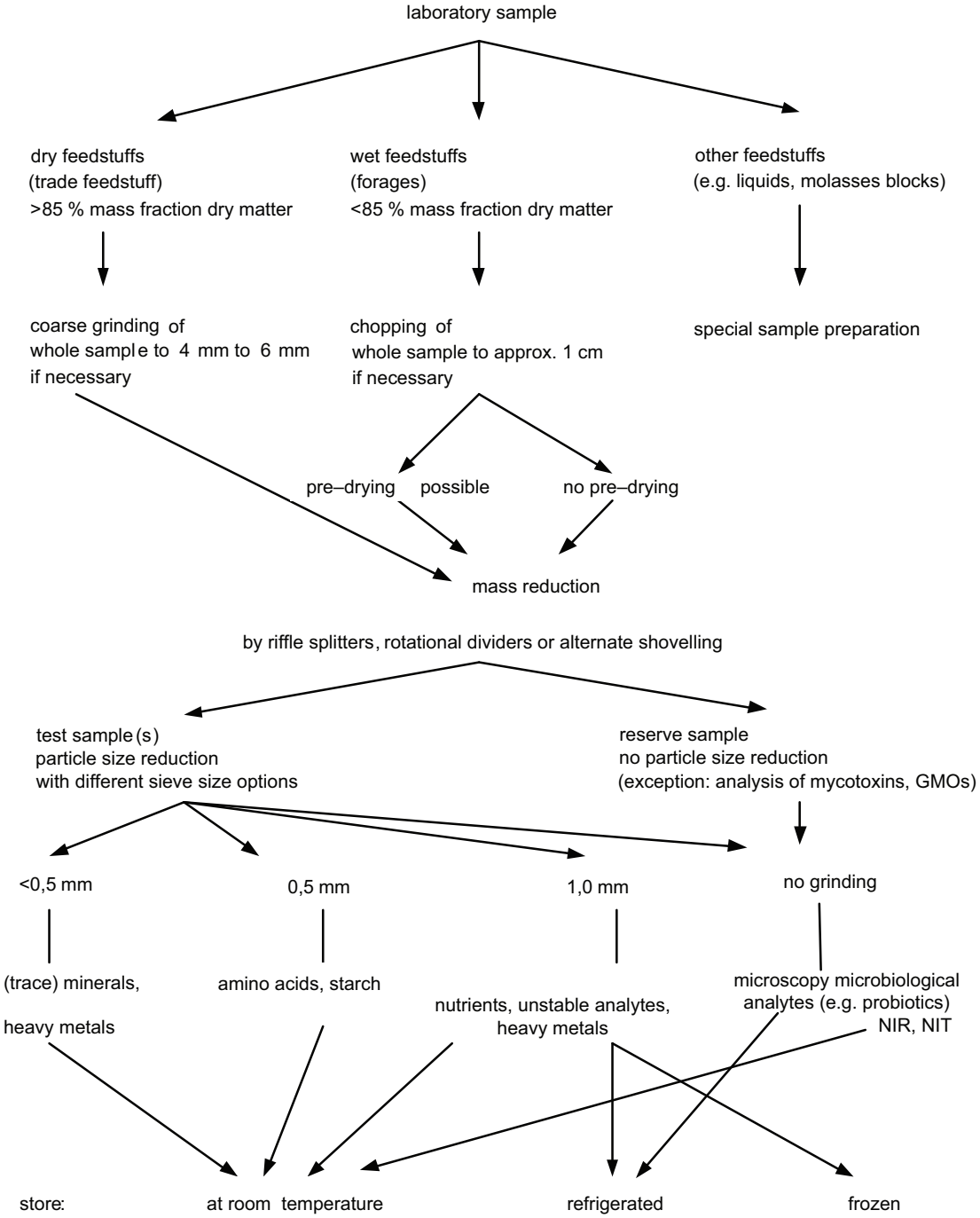


Figure 3 — Flowchart — Overview of sample preparation

9.2 Birdseed

The ingredients of birdseed have the tendency to separate due to differences in density and particle size of the assorted grains and oilseeds. Therefore, a rotary divider or a gated riffle divider is used to subsample birdseed. Save a reserve sample for microscopy or for an examination to detect the presence of noxious weed seeds. See Figure 4.

Grind test samples to a fine particle size to achieve uniform dispersal of all the ingredients. Oilseeds grind best in a blending-type mill or using cooling techniques such as dry ice.

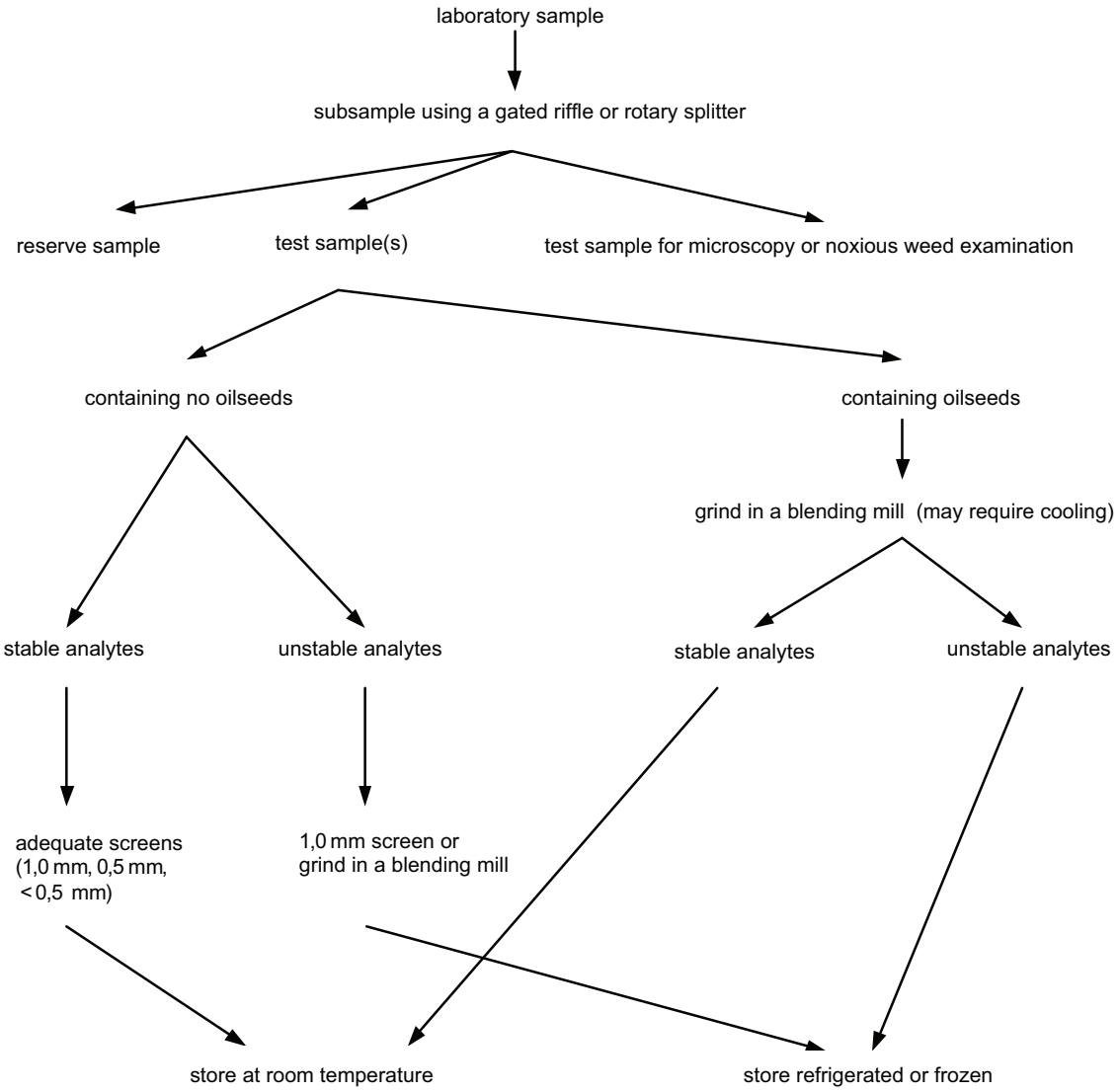


Figure 4 — Birdseed

9.3 Whole cottonseed

Whole cottonseed is received unprocessed (e.g. with the hulls and lint surrounding the meat). Due to difficulties in riffling caused by the non-uniformity of the lint-bearing seed, the entire laboratory sample is ground through a 6 mm screen. The remaining lint is then removed by hand by passing the meat through a 6 mm sieve.

The separated cottonseed meat is ground to a fine particle size. If mycotoxins are to be determined, an analytical subsample is stored frozen to prevent mould growth. A reserve of unground sample may be saved in case of a question concerning subsampling or grinding or a dispute over the results of the analysis.

Analyses are done on both the lint fraction and the meat fraction. The analytical results are combined mathematically based upon the mass fractions of total lint and total meat:

$$w_{\mathrm{W}} = \frac{w_{\mathrm{lint}} m_{\mathrm{lint}}}{m_{\mathrm{lint}} + m_{\mathrm{meat}}} + \frac{w_{\mathrm{meat}} m_{\mathrm{meat}}}{m_{\mathrm{lint}} + m_{\mathrm{meat}}} \tag{6}$$

where

w_W is the result for the whole cottonseed, expressed in grams per kilogram;

w_{lint} is the result for the lint fraction, expressed in grams per kilogram;

w_{meat} is the result for the meat fraction, expressed in grams per kilogram;

m_{lint} is the mass of the lint fraction, in grams;

m_{meat} is the mass of the meat fraction, in grams.

Take precautions to keep all equipment clean so as to avoid contaminating one sample with another. When necessary, wash equipment between samples. See Figure 5.

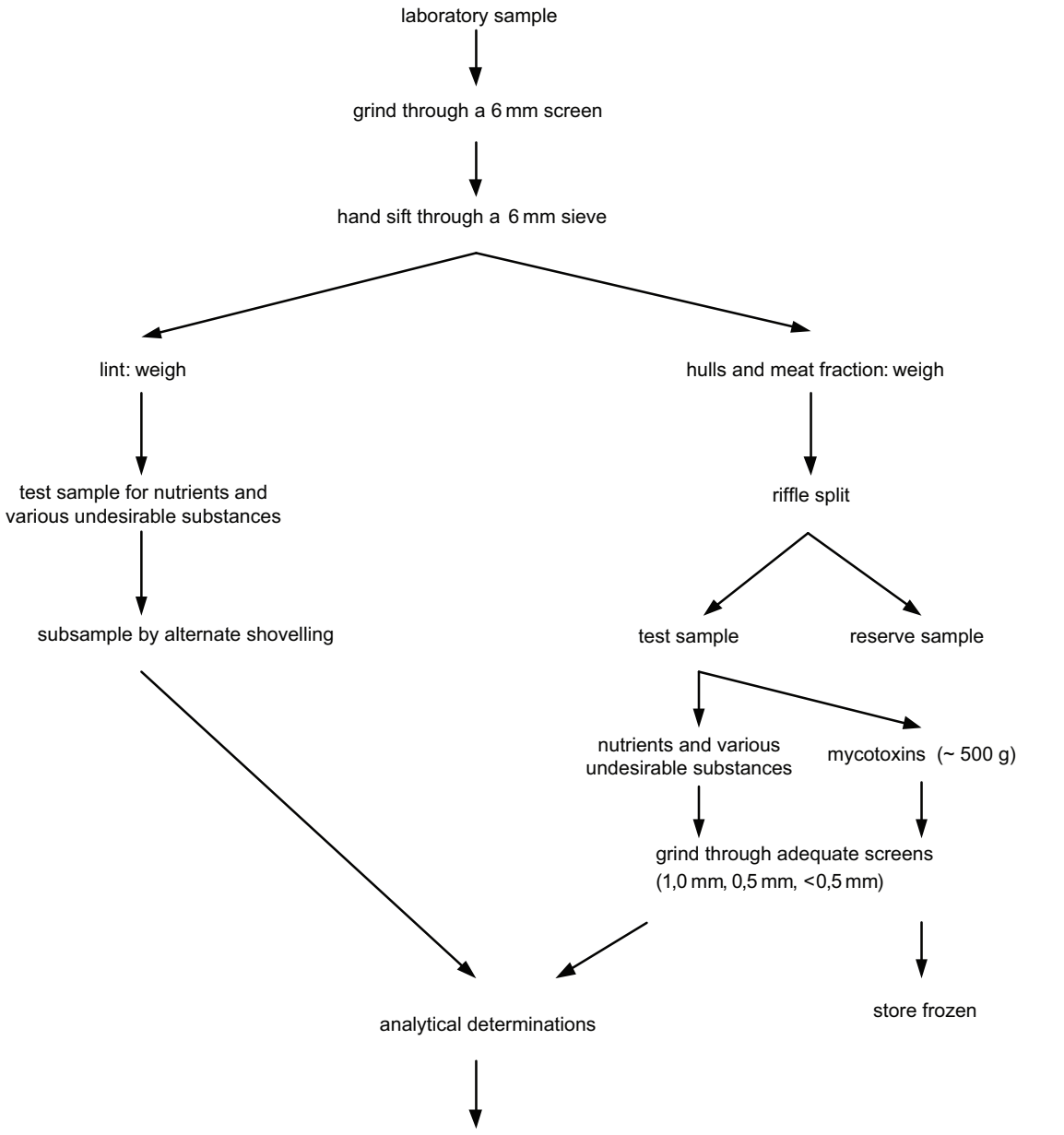


Figure 5 — Whole cottonseed

9.4 Mineral mix

Since differences in density and particle size may cause the components of mineral mixes to separate, it is advised that the mass be reduced using rotary or riffle dividers. The sample mass is reduced until the mass of the test sample is adequate. Save ungrounded reserve and test samples for microscopy and microbiological analysis (e.g. of probiotics), antibiotic, drug, and vitamin analysis at low temperatures since these analyses require large sample sizes.

Grind the test sample to a fine particle size for mineral analysis. Do not grind the drug, vitamin, microbiological analytes (e.g. probiotics) and antibiotic test sample(s) if the particle size distribution is adequate; otherwise, grind the test sample(s) on the day of analysis to a particle size of 1,0 mm. Rupturing, mixing and interaction of the ingredients with the heat of grinding can cause deterioration of vitamins, antibiotics and microorganisms (e.g. probiotics). Grinding also introduces air into the sample, causing oxidation. Refrigerate or freeze the vitamin test sample to prevent degradation.

Dividing or grinding a feed sample immediately after dividing or grinding a mineral mix creates a potential for contamination. Grouping samples by analyte content levels to avoid contamination is good practice. Process the feeds and lower-level mixes first. When necessary, wash equipment thoroughly whenever the potential for contamination exists. See Figure 6.

For microbiological analysis (e.g. of probiotics), the test sample should not be frozen.

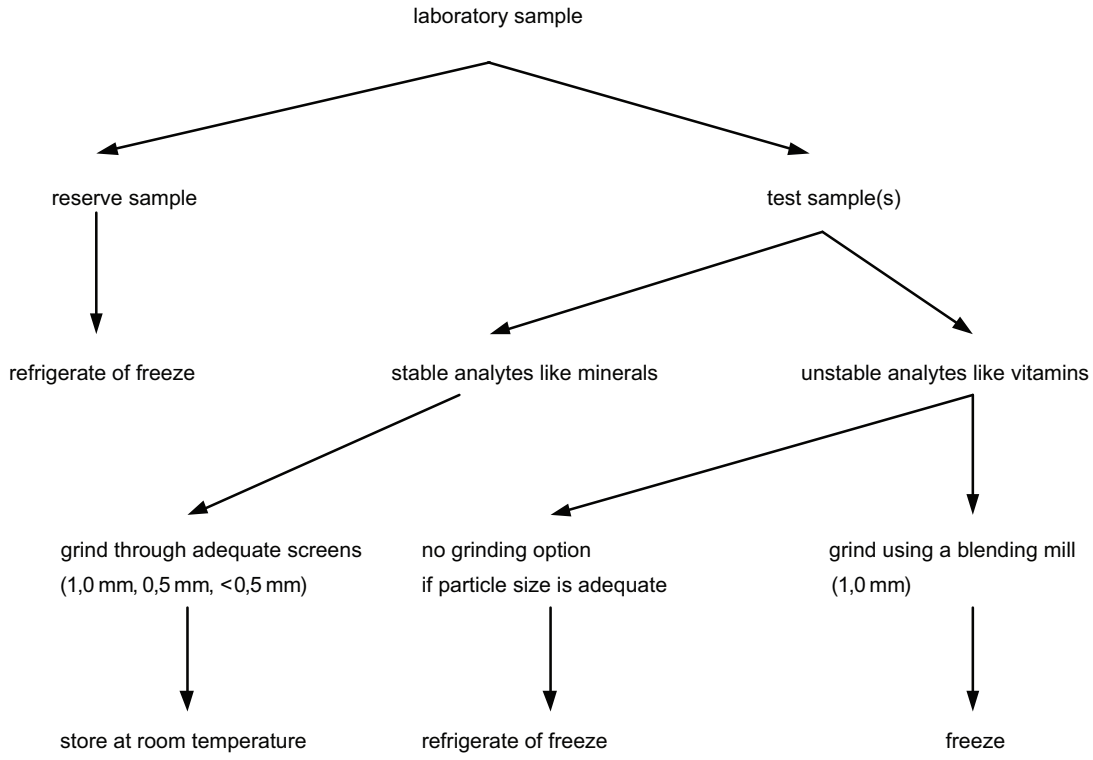


Figure 6 — Mineral mix

9.5 Dry feeds

Since differences in density and particle size may cause the components of dry feed to separate, use a riffle divider or a rotational divider to divide the sample into equal fractions. Save reserve ungrounded sample for microscopy or subsampling if required by laboratory policy. Save a separate container of unground laboratory sample for vitamin or drug analysis since this analysis requires large sample sizes.

Grind one of the test samples to a fine particle size for proximate and mineral analysis. Test samples for vitamins and antibiotics are not ground until the day of analysis. In the interval, to prevent degradation, refrigerate or

freeze vitamin and drug test samples. Rupturing, mixing and interaction of the ingredients with the heat of grinding expedites deterioration. Grinding also introduces air into the sample, causing oxidation.

For microbiological analysis (e.g. of probiotics) of feeds consisting of pellets which are easy to break down or of meal test samples are not ground; cereals and feeds consisting of pellets which are not easy to break down are coarsely ground without any heating. Test samples should not be frozen, but stored in a refrigerator in plastic bags with low microbe content and airtight closures.

Dividing or grinding a feed sample immediately after dividing or grinding a mineral mix, vitamin or drug premix creates potential for contamination. Grouping samples by analyte content levels to avoid contamination is a good practice. Finished feed should be processed before premixes or mineral mixes. When necessary, wash equipment thoroughly whenever the potential for contamination exists. See Figure 7.

Store samples in airtight containers to prevent a change in moisture content.

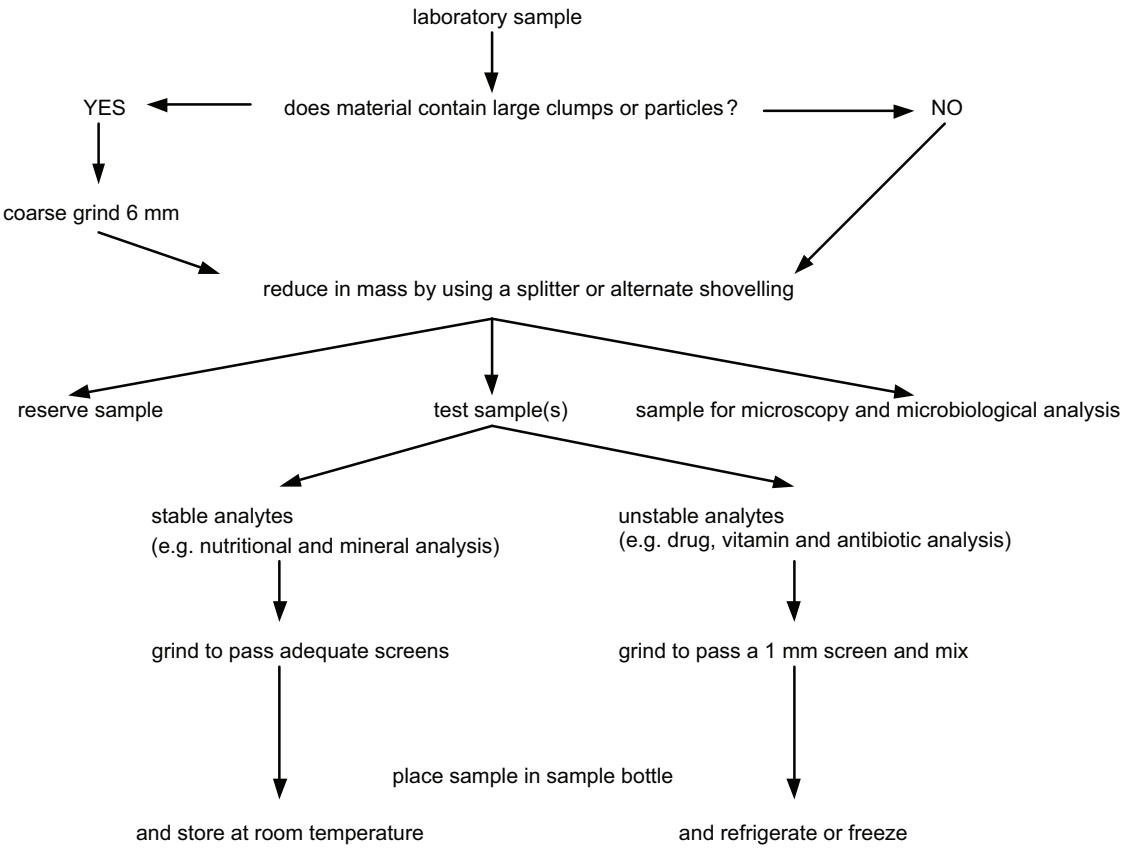


Figure 7 — Dry feeds

9.6 Forages including silage, hay, haylage, TMR and byproducts

Due to the diverse nature of forage materials, take care to obtain test samples of the laboratory sample. Coarsely grind and mix the entire sample. Save a reserve portion of the coarsely ground material in case of a question concerning sample reduction or analysis. Store dry reserve samples at room temperature; refrigerate or freeze wet reserve samples.

For some microorganisms (e.g. yeasts), freezing the sample can lead to degradation when the sample is defrosted; therefore, samples for microbiological analysis should only be stored in a refrigerator and not frozen.

Most forages, hay etc., received at a laboratory fall into one of the following categories:

— those dry enough to grind and analyse immediately (dry matter ≥90 %, e.g. grass or native hays, alfalfa pellets);

— those dry enough to be coarsely ground to pass a 4 mm to 6 mm sieve, but too wet to be finely ground (dry matter ≥85 %, e.g. legume hays);

— those samples which need to be partially dried before the sample can be coarsely ground (dry matter mass fraction <85 %, e.g. silages, fresh plants).

The “wetness” at which a forage material can be ground is determined by the forage material itself, the type of grinder to be used and the fineness of the grind. Most forages having a mass fraction of dry matter of greater than 85 % can be ground to pass a 4 mm to 6 mm sieve using a cutting mill without problems (sticking in the mill, moisture loss, etc.). However, when using a mill to grind forage material to pass a 1 mm sieve, most forages materials need to have a mass fraction of dry matter of greater than 90 % to be able to be properly ground.

Forage materials having a mass fraction of dry matter of approximately greater than or equal to 85 %, which are too large to grind in their entirety to the fineness required for analysis, are first ground through a cutting mill to pass a 4 mm to 6 mm sieve. Reduce the coarsely ground sample in a gated riffle divider or by alternate shovelling. When necessary, the partially dried and reduced analytical sample is ground to the fineness desired for analysis.

For carotene analysis, divide out a subsample after grinding through a 6 mm screen, regrind the subsample using a 1 mm screen, place in air tight container and freeze.

For mycotoxin analyses, use the entire laboratory sample when no other nutritional analysis are required. If both analyses are required, divide out a nutritional test sample after coarse grinding and use the reminder of the material for mycotoxin analysis. Weigh the entire laboratory sample or subsample into a dry, tared pan. Dry in a forced draft oven. Equilibrate to room temperature and weigh. Grind to pass a 1 mm screen. Transfer the entire ground sample to a large wide-mouth bottle and mix by tumbling for at least 5 min. It is necessary that the bottle be less than two-thirds full to allow for mixing.

Forage materials with dry matter mass fraction of less than 85 % need to be partially dried prior to grinding. The wet material is chopped, if needed, to facilitate drying and subsampling. The entire wet laboratory sample is dried, then coarsely ground to pass a 6 mm screen and then subsampled.

For microbiological analysis, samples are cut to pieces of 1 cm with a sterile cutter without drying.

Certain forage materials may require special handling to avoid loss of the analyte during sample preparation.

Cyanide (hydrocyanic acid) is rapidly released from chopped sorghum/Sudan grasses. Place a representative portion of the chopped wet laboratory sample in a plastic bag, seal, and freeze immediately. If whole plants are submitted from a grazing situation, sample only the portion which would be grazed.

Ammonia and organic acids (lactic acid, acetic acid, butyric acid) may be volatilized from ammoniated silages during oven drying. In this case, divide out a representative portion of the undried laboratory sample, place in a plastic bag, seal, and freeze when needed for protein, total nitrogen, non-protein nitrogen, ammonia or organic acid determination. See Figure 8.

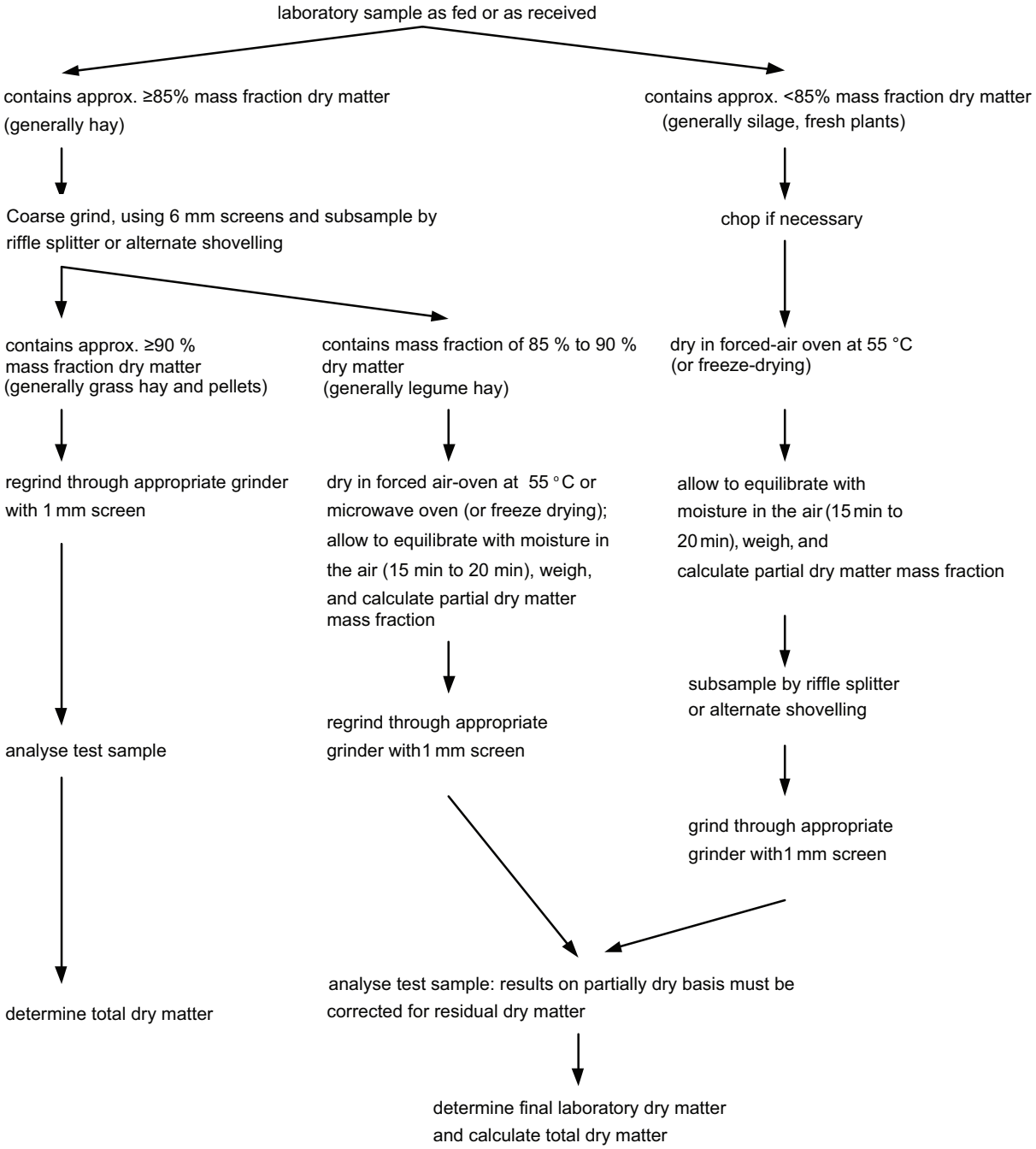


Figure 8 — Forages

9.7 Oilseeds and high-fat feeds

Oilseeds are easily subsampled using either a riffle divider or a rotational divider. Due to the oil content, it may be difficult to grind oilseeds in mills routinely used for dry feeds. Blending-type mills work well to grind oilseeds. Keeping the grinding chamber cold (by circulating coolant around it) or by grinding with dry ice helps to prevent the melting or the oxidation of fat during grinding. See Figure 9.

Subsampling hard fat and suet is extremely difficult since the fat particles cling to one another and to the equipment. Alternate shovelling is probably the best way to obtain a subsample. Blend hard fat (or suet) in a frozen state to prevent melting or oxidation of the fat. Use precautions during freeze-blending to prevent atmospheric moisture from condensing on the sample, which may alter the results. See Figure 10.

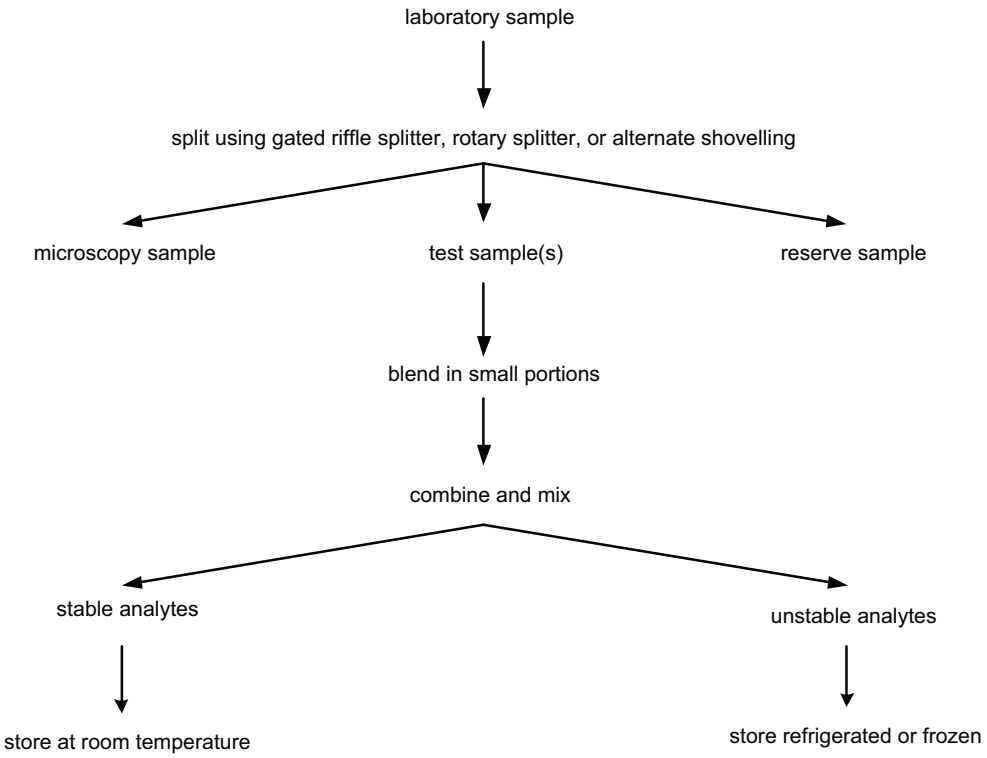


Figure 9 — Oilseeds and high-fat feeds

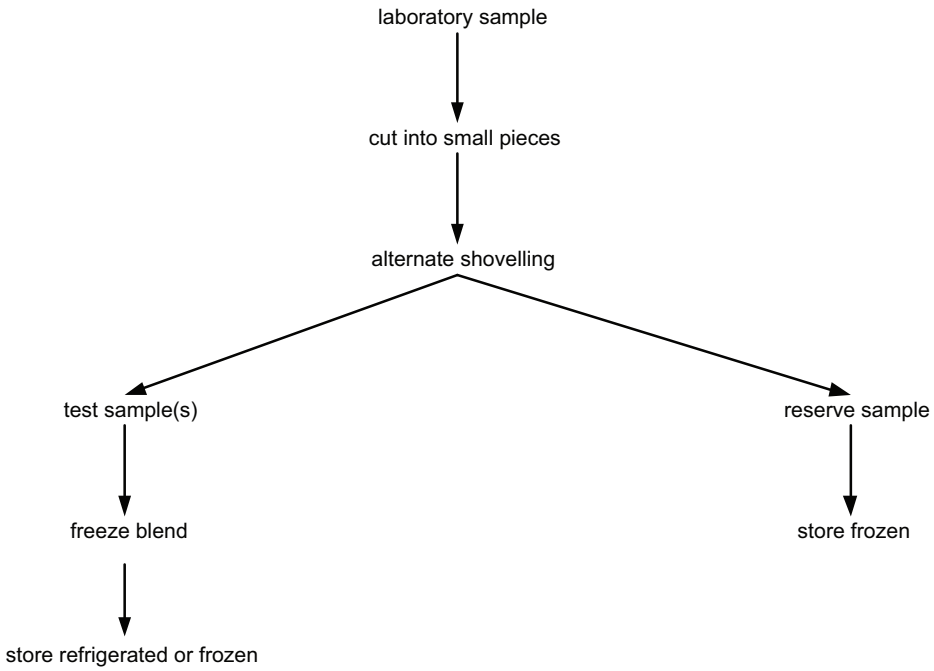


Figure 10 — Suet

9.8 Large block and molasses block feeds

Accurate subsampling from this sticky material is difficult.

For microbiological analysis, take a subsample by breaking it off before freezing.

A recommended approach is to place the entire laboratory sample in a freezer overnight. Use a mallet and break it into pieces small enough to be ground in a cutting mill. Using plenty of dry ice, grind the entire laboratory sample through the cutting mill using a 4 mm to 6 mm screen. Divide the laboratory sample using a riffle divider. Use generous amounts of dry ice during grinding and division to keep the laboratory sample cold enough to prevent clumping. Place the reserve sample in a plastic bag and store in a freezer. Regrind the analytical samples using a shearing mill or a blending mill and dry ice.

When using frozen laboratory samples, perform the entire process as quickly as possible to avoid clumping and condensation of atmospheric moisture on the sample, which alters the analytical results.

Process large feed and mineral blocks in a similar manner, omitting the freezing and dry ice steps, which are not needed.

Follow correct procedures for division and grinding so that the ground test samples are representative portions of the laboratory sample received. Take special care to affix the correct labels to the sample containers so that sample numbers are accurate. See Figure 11.

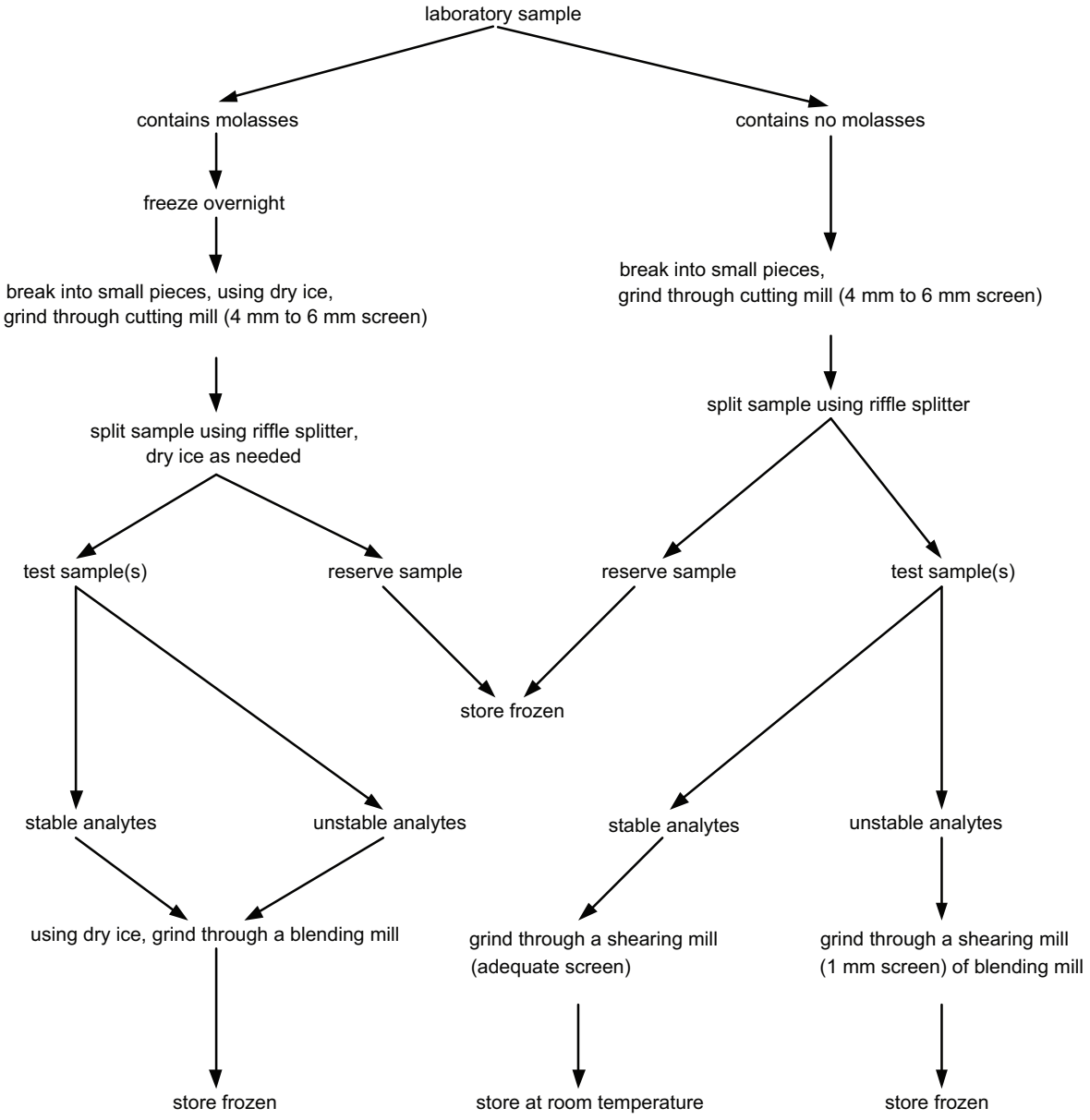


Figure 11 — Large block feeds and molasses block feeds

9.9 Liquid feeds

The ingredients of a feed have a tendency to separate due to differences in density and particle size. Therefore, a liquid feed sample is shaken, mixed or blended thoroughly before obtaining the test sample. The container needs to have sufficient space to allow mixing. Subsample during mixing or immediately following completion of mixing. All liquid feed samples are refrigerated to prevent degradation. For some microorganisms (e.g. yeasts), freezing the sample for storage can lead to degradation when the sample is defrosted. See Figure 12.

Freeze-drying is an option to reduce distribution error.

For microbiological analysis, the test sample should not be frozen. The analysis has to be carried out immediately.

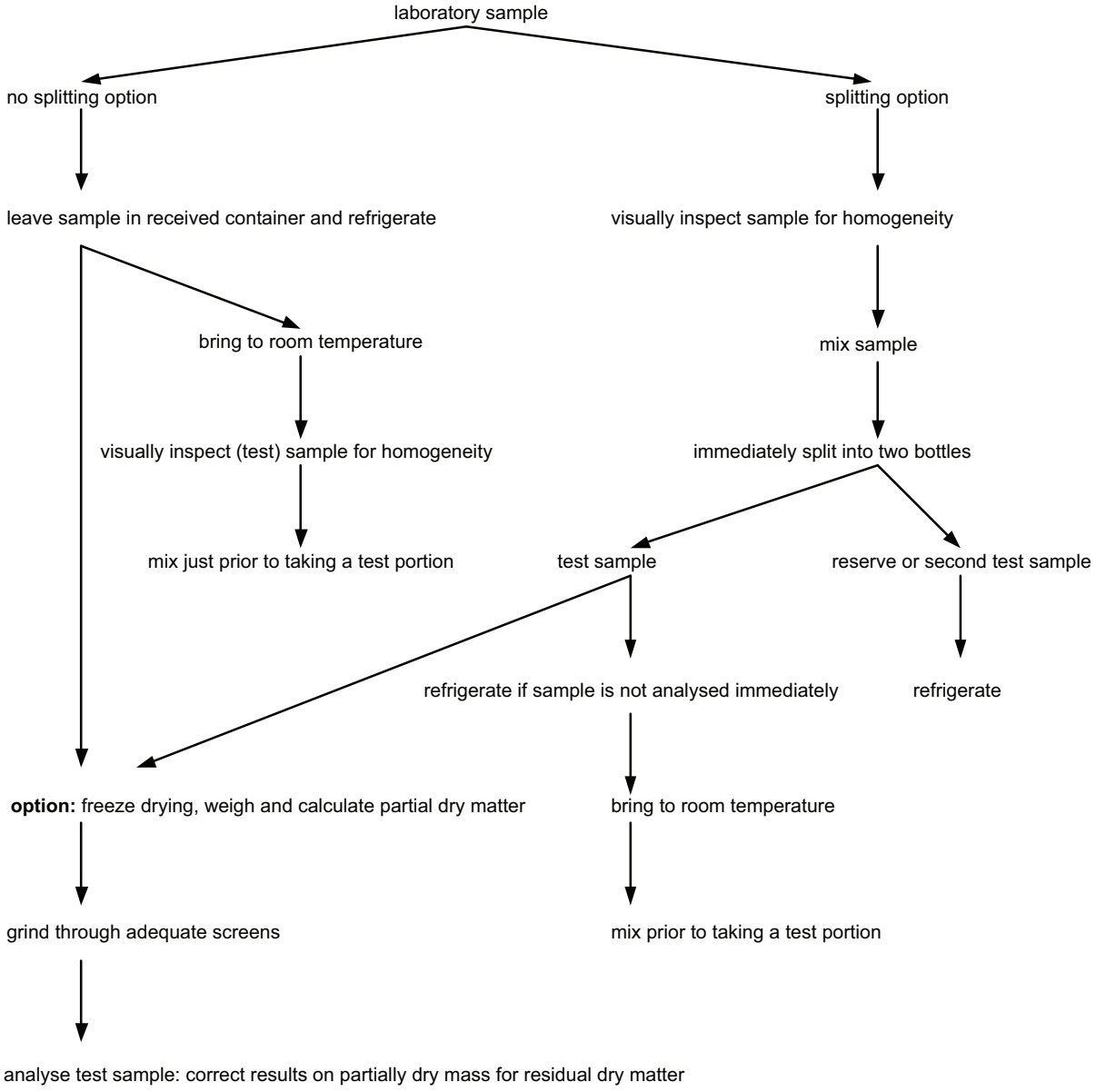


Figure 12 —Liquid feeds

9.10 Canned pet food

Take care when chopping and mixing the canned pet food material to include all of the liquid, fat, gelatin, and any other ingredients. Conduct sample preparation expeditiously to minimize the loss of moisture due to evaporation. Grind samples by blending in a food processor. The fat has a tendency to adhere to the walls of the bowl,

especially with a prolonged blending time. Therefore, blend in intervals of 30 s each and wipe down the walls between each interval. Blend high-fat-containing samples in a chilled state to prevent the fat from separating.

For microbiological analysis, a representative test sample is first removed under sterile conditions to avoid contamination of the opened container(s) with other microorganisms while preparing further test samples; do not freeze the test sample for microbiological analysis, but store in a refrigerator. See Figure 13.

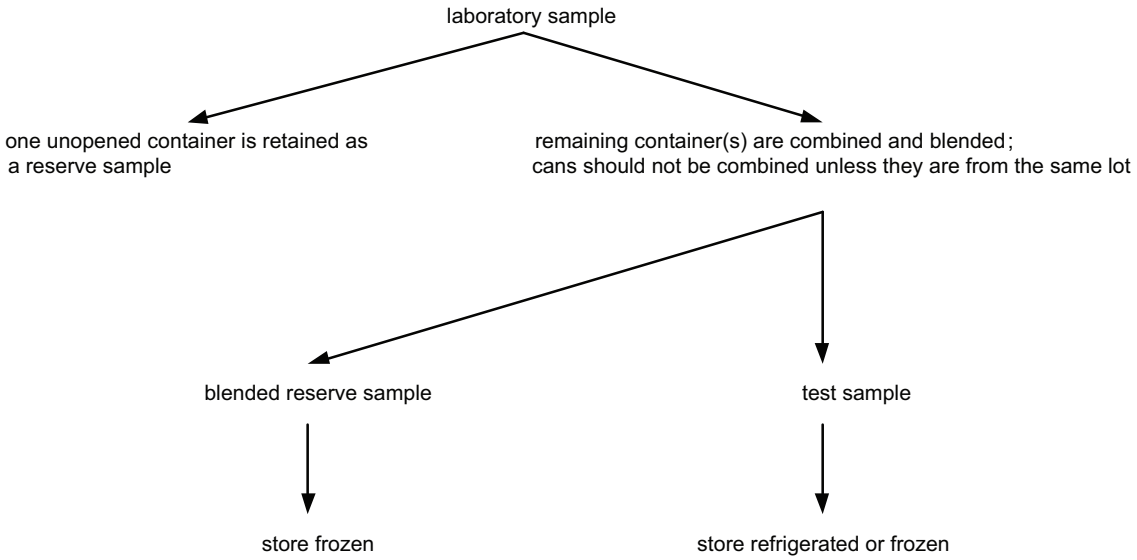


Figure 13 — Canned pet foods

9.11 Semi-moist pet food and dog chews

This tough leathery product grinds best in a mill that uses sharp knives, such as a cutting mill or a blender. The drier the material, the easier it is to grind. Moist material needs to be ground in a frozen state.

Subsampling is best done using either a gated riffle divider or by alternate shovelling.

Finely grind the analytical sample by either freeze-blending or using a cutting mill. Moist materials grind better by freeze-blending while dry materials can be ground in a cutting mill.

For microbiological analysis, coarsely grind the laboratory sample using a sterile cutter and do not freeze the corresponding test sample for grinding or storage purposes. See Figure 14.

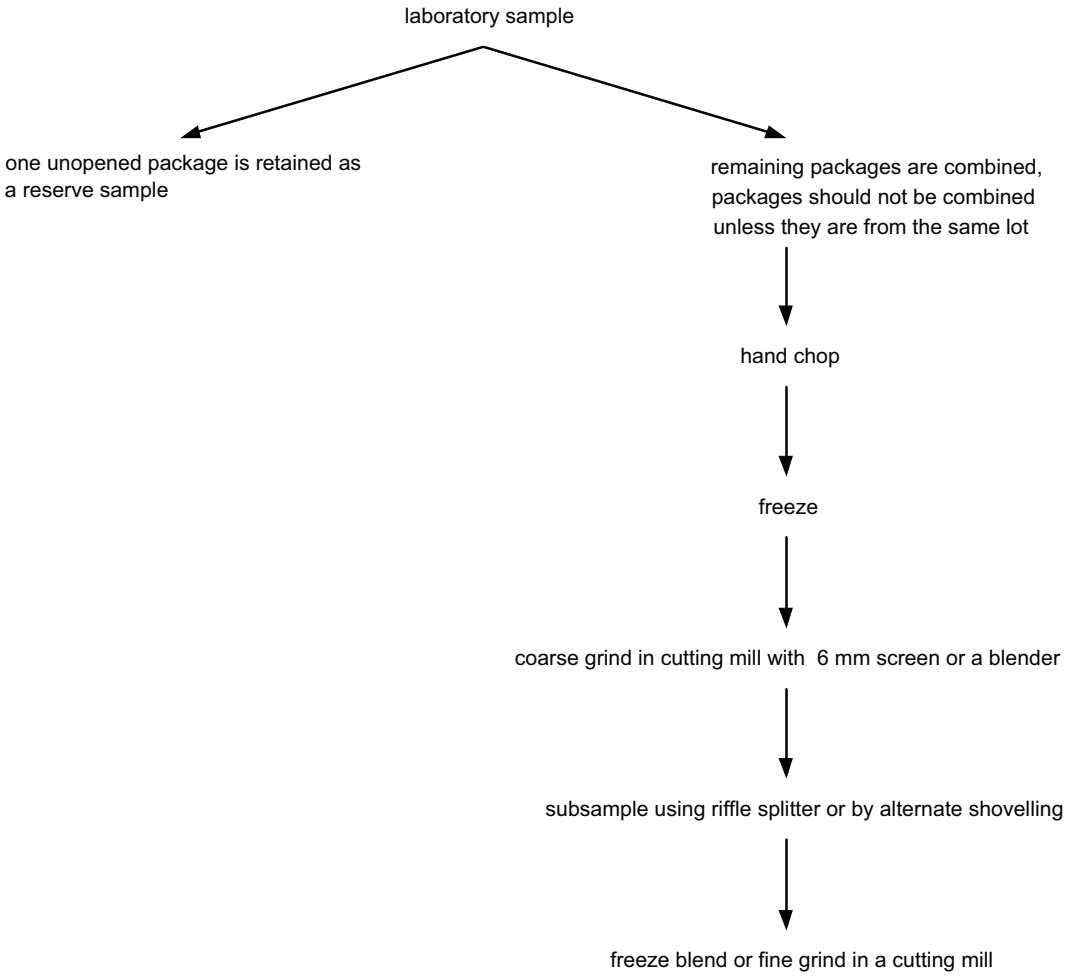


Figure 14 — Semi-moist pet food

9.12 Premixtures

Since differences in density and particle size may cause the components of premixes to separate, it is advised that the mass be reduced using rotational or riffle dividers. The sample mass is reduced until the mass of the test sample is adequate. Save a reserve sample if required by laboratory policy.

The premixes are generally of particle sizes that do not require grinding. If grinding is necessary, grind for a minimum amount of time to avoid heat generation. Rupturing, mixing and interaction of the ingredients with the heat produced by the grinding expedite deterioration. Grinding also introduces air into the sample, causing oxidation. Refrigerate or freeze the sample to prevent degradation. See Figure 15.

For microbiological analysis (e.g. of probiotics), the test sample should not be frozen.

Dividing or grinding a feed sample immediately after dividing or grinding a premix creates a potential for contamination. Grouping samples by analyte content levels to avoid contamination is good practice. Process feeds and the lower level mixes first. When necessary, wash equipment thoroughly whenever the potential for contamination exists.

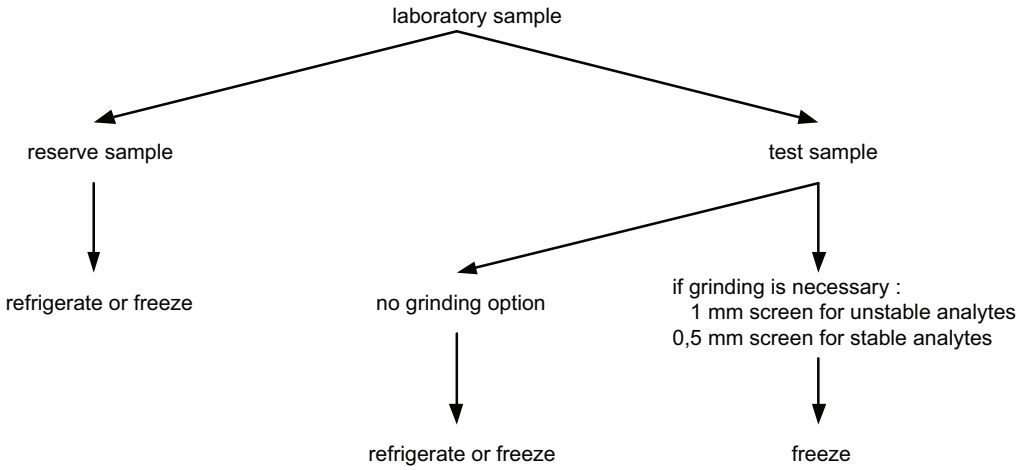


Figure 15 — Premixtures

9.13 Range and alfalfa hay pellets

To obtain a representative subsample from a bag containing large pellets and small particles (fines) is challenging. Randomly selecting three to six pellets for grinding, ignoring fines, from the 15 to 20 pellets submitted to the laboratory would be considered unacceptable practice.

Grind the entire laboratory sample including the fines through the cutting mill using a 4 mm to 6 mm screen. Using a 4 mm to 6 mm screen reduces the chance of heating and speeds up the process of grinding. Collect all the ground material, mix, and divide the sample into two or more representative portions using a riffle divider. Return the reserve portion of the ground sample to the original plastic bag and, if it contains heat-sensitive or degradable analytes, store in a freezer. Otherwise, store it at room temperature in an appropriate place. Finely grind the different test samples for stable and non-stable analytes. See Figure 16.

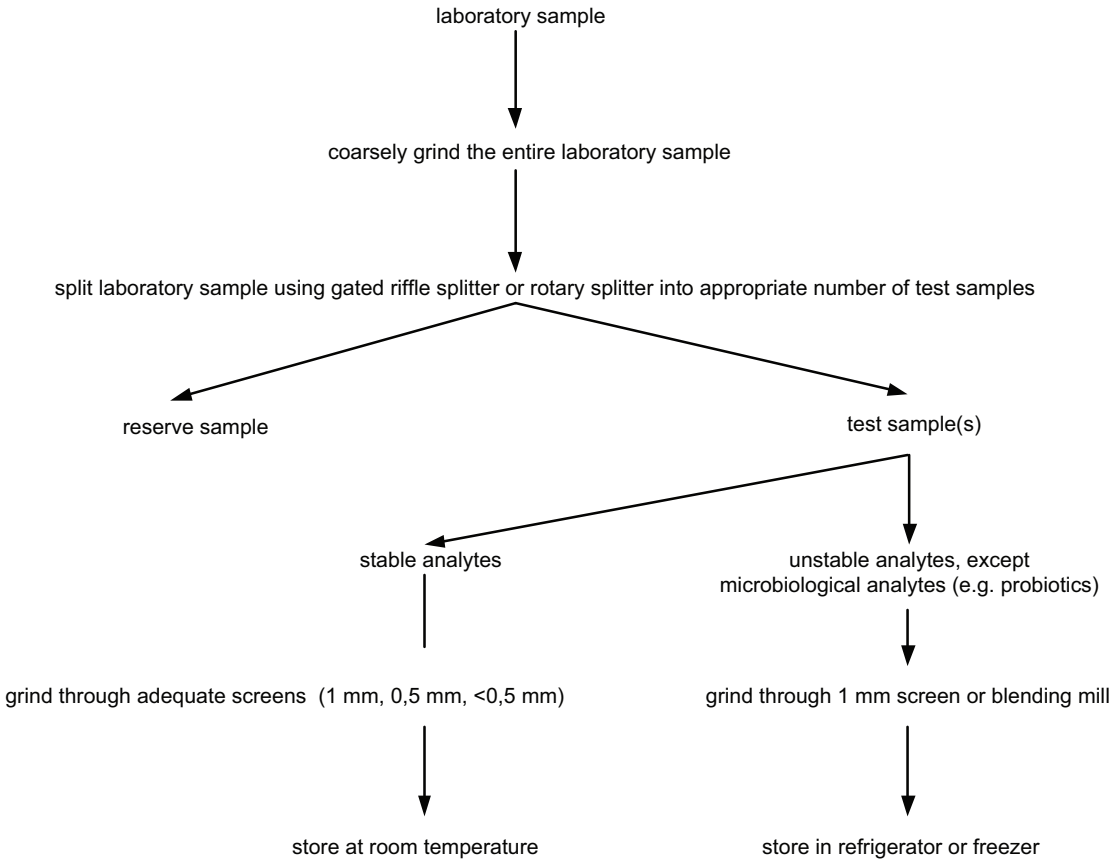


Figure 16 — Range and alfalfa hay pellets

9.14 Texturized and sticky feed

The sticky nature makes texturized feeds difficult to divide and grind. The grinding of a sticky material in a cold state helps to prevent the material from clumping together and reduces the potential for particles to adhere to the grinding equipment.

To obtain a representative subsample of a sticky feed, coarsely grind the feed first so that it passes through a 6 mm screen. For subsample feeds that are too sticky for a riffle divider, use the fractional shovelling technique.

Grind test samples to a fine particle size to achieve uniform dispersal of all ingredients. Feeds that are too sticky to grind in mills routinely used for dry feeds can be ground in blending-type mills.

Store test and reserve samples in airtight containers to prevent a change in moisture content. Use precautions during freeze-blending to prevent atmospheric moisture from condensing on the sample which may alter the results. See Figure 17.

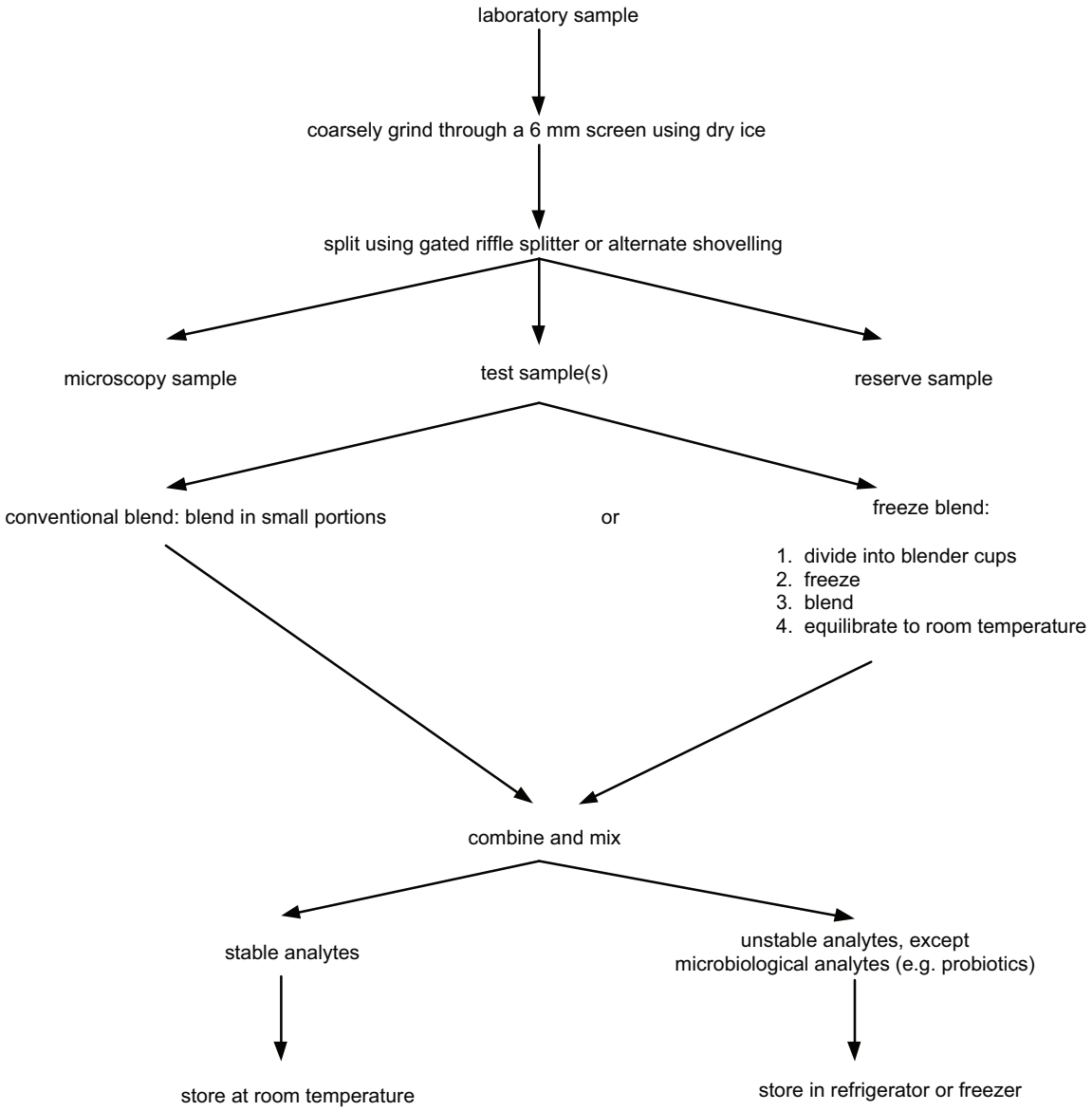


Figure 17 — Texturized and sticky feeds

9.15 Aquatic feeds

Grind one of the test samples to a fine particle size suitable for stable analytes, e.g. nutritional and mineral analysis. Test samples for vitamins and certain antibiotics are not ground until the day of analysis, these analyses require large test sample sizes. In the interim, to prevent degradation, refrigerate or freeze vitamin and drug samples. Rupturing, mixing and interactions of the ingredients with the heat produced by the grinding expedite deterioration. Grinding also introduces air into the sample, causing oxidation.

Test samples for microbiological analysis are not ground or frozen, only pelleted feeds which do not break down easily are shall undergo the coarse grinding process.

Store samples in airtight containers to prevent a change in moisture content. See Figure 18.

Figure 18 — Aquatic feeds

Annex A
(informative)

Calculations, examples and tables for minimum mass

The fundamental subsampling error (FSE) is the error that remains when the subsampling procedure is rid of incorrect errors and faults. This means that the FSE is the minimum subsampling error that can be obtained in practice and it is inherent only to the material heterogeneity. For this very reason it is, of course, method-independent. FSE can be calculated from a series of measurements as the difference between the estimate of the analyte content from measurements, w_S, and the actual analyte particle content in the laboratory sample, w_{LS}, from which the subsample is taken, i.e.

$$\frac{w_S - w_{LS}}{w_{LS}}$$

The variance of the FSE can be estimated to an order of magnitude using Gy's formula (see References [10][12]):

$$\sigma^2(\text{FSE}) = cfg\beta d^3\left(\frac{1}{m_{SS}} - \frac{1}{m_{LS}}\right) \tag{A.1}$$

where

c is the constitutional parameter, expressed in grams per cubic centimetre, that accounts for the densities as well as the proportions of the constituents;

f is the particle shape factor, dimensionless, describing the deviation from the ideal shape of a cube;

NOTE 1 For a cube, $f = 1$; for a sphere, $f = 0{,}52$; for spherical particles, $f = 0{,}5$; and for an almost flat disc, $f = 0{,}1$.

g is the size distribution factor, dimensionless, describing the range of particle sizes in the lot;

NOTE 2 For a wide size distribution, g = 0,25; for uniform particles, g = 1.

β is the liberation factor, dimensionless, describing the degree of liberation of the critical component from the matrix;

NOTE 3 For totally liberated particles, $\beta = 1$; for very small analyte particles incorporated in large particles of the matrix, $\beta = 0{,}03$. The liberation factor can be equated to $\sqrt{(L/d)}$, where L is the particle size of the analyte particles "trapped" in the matrix particles of particle size d. For $\beta = 0{,}03$ with a matrix particle size $d = 0{,}01$ cm, this corresponds to an analyte particle size of ~10 µm (molecular level).

d is the top particle size, defined as the square-mesh screen that retains 5 % of the material (length expressed in centimetres);

m_{SS} is the mass of the subsample;

m_{LS} is the mass of the laboratory sample from which the subsample is taken.

A more detailed description of the constitutional parameter, c, is:

$$c = \frac{(1 - w_{LS})^2}{w_{LS}} \rho_c + (1 - w_{LS}) \rho_m \quad \text{(A.2)}$$

where

w_{LS} is the content of the analyte particles present in the laboratory sample;

ρ_c is the density of the analyte particles;

ρ_m is the density of the matrix.

Gy's formula was derived from mineralogical samples and works for binary mixtures of particulate material where the particles sought (i.e. the analyte particles) are present as separate fragments. It therefore only produces approximate results for feeding stuffs, but gives a high-end estimate of the fundamental sampling error as well as an indication of the dependence on particle size and sample mass.

Three examples of the minimum sample mass needed for set FSE (% CV) and particle size values are given below. It should be noted that using appropriate mass-reducing devices, e.g. riffle dividers or vario dividers, generally leads to lower values of the FSE than can be calculated from Gy's formula, whereas grab sampling can give rise to even higher values of FSE (see References [10][12]).

EXAMPLE 1 To a mineral mixture (ρ_m = 1,5 g/cm^3) is added 0,4 % mass fraction methionine as DL-methionine sulfoxide ($\rho_c \approx$ 2 g/cm^3). As the analyte particles are completely liberated, $\beta = 1$. Due to a wide range of particle sizes, g = 0,25. The sample mass, m_{LS}, is 100 g and the minimum mass of the subsample, m_{SS}, depending on the tolerable FSE can be seen from Table A.1. $C = cfg\beta$ in the text below.

EXAMPLE 2 To a mineral premixture (ρ_m = 2 g/cm^3) is added 10 mg/kg Se as Na_2SeO_3 (ρ_c = 3,1 g/cm^3). As the analyte particles are completely liberated, $\beta = 1$. Due to a wide range of particle sizes, g = 0,25. The sample mass, m_{LS}, is 100 g and the minimum mass of the subsample, m_{SS}, depending on the tolerable FSE can be seen from Table A.2.

EXAMPLE 3 An organic feeding stuff ($\rho_m = \rho_c$ = 0,8 g/cm^3) contains 10 mg/kg Cu as a natural component. Since the analyte is completely incorporated into the matrix particles (best-case scenario), β = 0,03. Due to a wide range of particle sizes, g = 0,25. The sample mass, m_{LS}, is 100 g and the minimum mass of the subsample, m_{SS}, depending on the tolerable FSE can be seen from Table A.3.

Variable	Example 1	Example 2	Example 3
w_{LS}, %	0,4	0,001	0,001
ρ_c, g/cm^3	2	3,1	0,8
ρ_m, g/cm^3	1,5	1,5	0,8
m_S, g	See Table A.1	See Table A.2	See Table A.3
m_{LS}, g	100	100	100
c	498	309 995	79 999
f	0,5	0,5	0,5
g	0,25	0,25	0,25
β	1	1	0,03
C	62	38 749	300

Equation (A.3) is only an approximation, therefore the variables do not have to be measured precisely. In most cases, an estimation of variables is appropriate.

$$m_S = 10 \times \frac{\rho d^3}{\hat{C}_V^2} \quad \text{(A.3)}$$

where

m_S is the minimum mass, in grams, of the sample collected;

$\hat{C}_V$ is the tolerable error (fundamental subsampling error), expected coefficient of variation (CV);

d is the size, in centimetres, of the largest particle;

ρ is the density of the material, in grams per cubic centimetre.

EXAMPLE 4 If the maximum particle size is 4 mm and the density is 0,8 g/cm^3 and the tolerable error is 15 % CV, the minimum mass needed to represent all the particle sizes for this error is 23 g.

Table A.1 — Minimum mass of a subsample when methionine is added to a mineral mixture

d, mm	**FSE (expected CV)**, %				
	20	15	10	5	2
	Minimum sample mass, g				
0,1	0,002	0,003	0,01	0,02	0,2
0,2	0,01	0,02	0,05	0,2	1
0,5	0,2	0,3	0,8	3	16
1	2	3	6	20	61

Table A.2 — Minimum mass of a subsample when selenium is added to a mineral premixture

d, mm	**FSE (expected CV)**, %				
	20	15	10	5	2
	Minimum sample mass, g				
0,1	1	2	4	13	49
0,2	7	12	24	55	89
0,5	55	68	83	95	99
1	91	95	97	99	100

Table A.3 — Minimum mass of a subsample when copper is a natural component of an organic feeding stuff

d, mm	**FSE (expected CV)**, %				
	20	15	10	5	2
	Minimum sample mass, g				
0,1	0,01	0,01	0,03	0,1	1
0,2	0,1	0,1	0,2	1	6
0,5	1	2	4	13	48
1	7	12	23	55	88

The error of a particular mass can also be calculated from:

$$\hat{C}_V^2 = 10 \times \frac{\rho d^3}{m_S} \tag{A.4}$$

The largest adequate particle size can also be calculated:

$$d = \left(\frac{\hat{C}_V^2 m_S}{10\rho} \right)^{1/3} \tag{A.5}$$

EXAMPLE 5 A laboratory sample has a largest particle size of approximately 2 mm, a density of approximately 0,7 g/cm^3 and weighs 1 kg. Without grinding and with a tolerable error of 5 %, a minimum mass, m_S, of 22 g is required for analysis.

$$m_S = 10 \times \frac{0,7 \times 0,2^3}{0,05^2} = 22 \tag{A.6}$$

If the analytical procedure requires only 1 g, the FSE is 24 % without grinding.

$$\hat{C}_V^2 = 10 \times \frac{0,7 \times 0,2^3}{1} = 0,056$$
$$\hat{C}_V = 0,24 \tag{A.7}$$

Therefore, if the desired laboratory subsampling error is not to be more than 5 % CV, the sample would have to be ground to a particle size of 0,07 cm = 0,7 mm (or less).

$$d = \left(\frac{0,05^2 \times 1}{10 \times 0,7} \right)^{1/3} = 0,07 \text{ cm} = 0,7 \text{ mm} \tag{A.8}$$

Bibliography

[1] ISO 664:2008, *Oilseeds — Reduction of laboratory sample to test sample*

[2] ISO 6497:2002, *Animal feeding stuffs — Sampling*

[3] Commission Regulation (EC) No. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* 2009-02-26, **L54**, pp. 1–130

[4] Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Off. J. Eur. Union* 2002-02-01, **L31**, pp. 1–24

[5] Regulation (EC) No. 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed. *Off. J. Eur. Union* 2009-09-01, **L229**, pp. 1–28

[6] Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. *Off. J. Eur. Union* 2003-10-18, **L268**, pp. 29–43

[7] AAFCO Laboratory Methods and Service Committee. *Guidelines for preparing laboratory samples*, 2nd edition. Association of American Feed Control Officials Incorporated, 2003

[8] Standards Commission for Straight Feeding Stuffs. *Positive list for straight feeding stuffs*, 9th edition. Berlin: Central Committee of German Agriculture, 2010, 49 p. Available (viewed 2012-01-03) at: http://statictypo3.dlg.org/fileadmin/downloads/fachinfos/futtermittel/positivliste/positivlist_en_9.pdf

[9] VDLUFA. Chapter 2, Behandlung der Versandmuster und Herstellung der Analysenprobe [Handling of laboratory samples and preparation of test portions]. In: *Methodenbuch* [Analytical methods manual], Vol. 3, *Die chemische Untersuchung von Futtermitteln* [The chemical investigation of animal feeds]. Speyer: Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

[10] Minkkinen, P. Practical applications of sampling theory. *Chemometr. Intell. Lab. Syst.* 2004, **74**, pp. 85–94

[11] Petersen, L., Dahl, C.K., Esbensen, K.H. Representative mass reduction in sampling — A critical survey of techniques and hardware. *Chemometr. Intell. Lab. Syst.* 2004, **74**, pp. 95–114

[12] Petersen, L., Minkkinen, P., Esbensen, K.H. Representative sampling for reliable data analysis. *Chemometr. Intell. Lab. Syst.* 2005, **77**, pp. 261–277

[13] Allen, T., Kahn, A.A. Critical evaluation of powder sampling procedures. *Chem. Eng. (London)* 1970, **238**, pp. CE108–CE112

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