International Standard 6498

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Animal feeding stuffs — Preparation of test samples

Aliments des animaux — Préparation des échantillons pour essai

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 6498 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1981.

It has been approved by the member bodies of the following countries :

Australia	Israel	Romania
Austria	Italy	South Africa, Rep. of
Brazil	Kenya	Spain
Canada	Korea, Dem. P. Rep. of	Sri Lanka
Chile	Korea, Rep. of	Tanzania
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Ethiopia	Netherlands	Turkey
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The member body of the following country expressed disapproval of the document on technical grounds :

United Kingdom

Animal feeding stuffs — Preparation of test samples

1 Scope

This International Standard specifies methods for the preparation of test samples of animal feeding stuffs.

2 Field of application

It is applicable to all the animal feeding stuffs listed in tables 1 to 4. For the analysis of special characteristics (microbiological, toxicological, etc.), special instructions will be given in the appropriate International Standards.

3 References

ISO 5986, *Animal feeding stuffs — Determination of diethyl ether extract.*

ISO 6496, *Animal feeding stuffs — Determination of moisture content.*

ISO 6497, *Animal feeding stuffs — Sampling.*[1]

4 Definitions

For the purpose of this International Standard, the following definitions apply.

laboratory sample; final lot sample; submitted sample : A sample representative of the quality and condition of the lot, obtained by reduction of the bulk sample and intended for analysis or other examination.

sample for analysis : A representative portion of the laboratory sample, obtained by dividing by means of a sample divider or by hand, if necessary after reduction of the particle size.

test sample : A representative portion of the sample for analysis or laboratory sample, obtained by reduction of the particle size, if necessary after dehydrating or de-fatting.

5 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

5.1 Reagents for de-fatting

5.1.1 Diethyl ether, anhydrous, virtually free from peroxides (ϱ_{20} = 0,720 g/ml, boiling point 34 to 35 °C) and having a residue on evaporation of less than 5 mg/100 ml.

5.1.2 ***n*****-hexane** or, failing this, **light petroleum** distilling between 40 and 60 °C and having a bromine value of less than 1. For either solvent, the residue on evaporation shall not exceed 2 mg/100 ml.

5.1.3 Acetone.

5.1.4 Carbon tetrachloride, having a residue on evaporation of less than 5 mg/100 ml.

5.1.5 Silicon carbide chips.

5.2 Reagent for drying

Diatomaceous earth, boiled for 30 min in 6 mol/l hydrochloric acid solution, washed with water until acid-free, and dried at 130 °C.

6 Apparatus

Usual laboratory apparatus, and

6.1 Apparatus for sample division and reduction

6.1.1 Sample divider, for example a multiple slot divider with a sorting system (see figure 1) or a conical divider (see figure 2) or a rotating distributor.

1) At present at the stage of draft.

6.1.2 Knife, scissors or mechanical cutting device.

6.1.3 Grater.

6.1.4 Grinder, suitable for producing particles which will pass through a sieve of nominal aperture size 1,0 mm or, failing this, a **mortar**.

6.1.5 Sieves, of nominal aperture sizes 1,0 and 2,0 mm.

6.2 Apparatus for de-fatting

6.2.1 Soxhlet-type extraction apparatus, or equivalent.

6.2.2 Electrically heated oven, well ventilated, capable of being controlled at 103 ± 2 °C.

6.2.3 Desiccator, containing an efficient desiccant.

6.2.4 Balance, accurate to 1 mg.

6.3 Apparatus for drying

6.3.1 Electrically heated oven, well ventilated, capable of being controlled at 70 ± 2 °C and at up to 100 °C, or

6.3.2 Electrically heated vacuum oven, capable of being controlled at 80 ± 2 °C and of reducing the pressure to less than 13,3 kPa (100 mmHg), fitted with a device for the introduction of dry air or containing a desiccant, for example calcium oxide.

6.3.3 Trays, of aluminium, enamel or stainless steel.

NOTE — Plastics trays have a tendency to absorb moisture.

6.3.4 Balance, accurate to 0,1 g.

7 Sampling

See ISO 6497.

8 Procedure

8.1 Laboratory sample

8.1.1 Quantity

The required quantity of sample depends on the nature of the product, in particular its particle size and homogeneity. The minimum quantities required for laboratory samples of various products are given in tables 1 to 4.

Table 1 — Roughages

Product	Minimum size of laboratory sample, g
Undried green fodders, such as fresh grass, lucerne, beanstraw, leaves of beet and tubers, etc. Undried potatoes, roots, etc. Tubers, beets, turnips, etc. Silages Wet by-products Dried corncobs, etc.	1 000
Hay, straw, dried grass, lucerne hay, etc. Dried pulp, potato pulp, etc.	500

Table 2 — Concentrates

Product	Minimum size of laboratory sample, g
(By-)products from milling and peeling industries (cereals) starch industry (potato flakes) fermentation industry, sugar industry Compound feeds, dairy products	500
Products of animal origin Products of oil and fat industries Tapioca roots and similar products	1 000

Table 3 — Cereals, pulses and seed

Product	Minimum size of laboratory sample, g
Cereals, pulses Oilseeds (small sizes such as linseed and rapeseed) and other seeds and fruits (small sizes such as carob kernels and juniper berries)	1 000
Oilseeds of large sizes (such as soyabeans and sunflower seeds) and other seeds and fruits of large sizes (such as acorns and olive stones)	2 500
Copra and similar products	6 000

Table 4 — Other products

Product	Minimum size of laboratory sample, g
Vitamin concentrates, minerals, oils and fats	250
Molasses, distillers solubles, fish solubles and other liquids	1 000

8.1.2 Storage

Store the laboratory sample in such a way that changes in composition are avoided. Store spare samples in the same way. For easily perishable samples, drying prior to storage, or cold storage, may be necessary.

8.2 Sample for analysis

8.2.1 Size

The size of the sample for analysis shall be at least 100 g expressed on the dry matter basis. If the laboratory sample does not exceed 500 g of dry matter, this shall be considered as the sample for analysis. If the laboratory sample exceeds 500 g of dry matter, its size shall be reduced as described in 8.2.2.

8.2.2 Preparation

If necessary, subsampling shall be carried out using a sample divider (6.1.1) or by hand. Prior to subsampling, reduce, if necessary, the particle size by means of a knife or scissors to pieces of length about 30 mm or using the mechanical cutting device (6.1.2).

8.2.2.1 For sampling solid, non-milled samples, such as roughages, by hand, proceed as follows.

Empty the laboratory sample onto a plastic or rubber sheet. Take, starting from one side, a portion of the sample, ensuring that nearly all particles from top to bottom are included in the portion. Spread it in a thin layer on a second sheet. Take a second portion from the laboratory sample in the same way and spread it over the first layer. Continue until the whole sample has been transferred to the second sheet. Finally, spread all remaining particles from the first sheet over the sample on the second sheet. Repeat the whole procedure twice more.

To obtain the sample for analysis from the well-mixed product proceed as follows.

Take many small samples from various places of the heap, ensuring that, in each case, all particles, from top to bottom, are included, until the sample for analysis is obtained.

8.2.2.2 Laboratory samples of milled products shall be carefully mixed and divided. If a sample divider is not available, the sample for analysis shall be obtained by taking many small amounts with a spoon from many places in the mixed sample. Take care that segregation does not adversely affect sampling.

8.2.2.3 If subsampling beets or similar products, take a slice from top to bottom of each beet and grate it.

8.2.2.4 For homogeneous products, the quartering technique may be used instead of the procedures described above. Proceed as follows.

Thoroughly mix the product and heap it to form a "cone". Then flatten and quarter it. Reject two diagonally opposite quarters. Carefully mix the remainder and continue the quartering and rejection until the sample has been reduced to the desired size.

8.2.2.5 In the case of liquid or pasty products, if necessary heat pasty products to 40 to 50 °C, then stir the laboratory sample well and divide it manually by pouring into containers of capacity about 100 ml.

NOTE — All operations should be carried out in such a way that the composition of the sample, including its moisture content, is not changed.

8.3 Test sample

8.3.1 Size

The whole sample for analysis shall be used for the preparation of the test sample.

8.3.2 Samples having low moisture contents

8.3.2.1 Particle size

The particle size of the sample shall be such that the whole sample passes through a sieve of nominal aperture size 1 mm. If the sample is coarse, carefully grind it until it completely passes the sieve. If the grinder does not have such a sieve, or if grinding is not carried out in a grinder, sieve the sample for analysis on the sieve (6.1.5) of nominal aperture size 1 mm. If the sample is fine and passes through the sieve, mix carefully and transfer it to a corrosion-proof container provided with an airtight closure and store in a dark, cool place.

Samples of products which cannot be conveniently ground or sieved shall be prepared by other suitable means.

8.3.2.2 Grinding

Grinding shall be carried out as quickly as possible with minimum exposure of the sample to air. The use of a mortar is often permissible, but is slower than a grinder.

Grinding may result in an unacceptable loss or gain of moisture and in a loss of volatile matter; allowances shall be made for such effects. (See 8.3.3 for samples having high moisture contents.)

It should be noted that some fine material, such as dust, may be lost and/or that hard particles may be ejected; such losses shall be prevented as much as possible.

If the sample is very unstable to heat or air, grinding may be restricted to a particle size of 2 mm or omitted altogether. Care shall be taken to ensure that the grinder itself is not a possible source of contamination (such as iron, copper, chromium).

If the test sample cannot be easily ground because of its moisture or fat content, continue as described in 8.3.3, 8.3.4 or 8.3.5.

8.3.3 Samples having high moisture contents and which are difficult to grind

Pre-drying of the sample is carried out to improve the physical, chemical and biological stability of the sample during grinding and storage. The drying process may induce some chemical

and biological changes in the product. In general, safe drying is assured if the following procedure is used.

Spread the sample for analysis (m_0) in a thin layer on a tray (6.3.3), weigh to the nearest 0,1 g, and place in the oven (6.3.1), controlled at 100 °C. As soon as the temperature of the sample is assumed to be 80 °C, switch on the ventilation and set the temperature to 70 °C. If no alteration in composition is expected, the sample may be placed directly in the ventilated oven at 70 ± 2 °C.

The drying time shall be the minimum required to produce a sample having a moisture content of about 7 % (m/m) after reaching equilibrium with air. The drying time should normally be 24 h, and, after intervals of 1 h and 12 h, the sample shall be turned over on the tray. If oven drying is likely to produce changes in composition, either drying in the vacuum oven (6.3.2) at a lower temperature, or freeze drying, may be performed. Such methods may result in a loss of volatile matter.

Condition the sample in the ambient atmosphere for 2 h, preferably in the room where grinding is to take place.

Weigh the tray and sample (m_1), to the nearest 0,1 g, and grind the sample immediately in a grinder to give particles of size less than 1 mm. Avoid, as much as possible, the loss of fine particles.

Determine the moisture content of the prepared sample so as to be able to correct the results of analyses to correspond to the original moisture content of the sample.

The loss of volatile matter by evaporation, for example in the case of silages, can be corrected by determining the volatile matter in the fresh sample and in the sample after drying and grinding.

NOTE — Molasses should not be ground, but should be analysed in the fresh state. If the sample has to be dried, proceed as described in 8.3.3.

8.3.4 Samples having high fat and low moisture contents and which are difficult to crush

Roughly grind, grate or crush the required quantity of sample. Some products may be coarsely ground, taking care that the sample does not become greasy.

Soft oilseeds (such as groundnuts and copra) shall preferably be grated; hard oilseeds (such as palm kernels and soya beans) shall be ground in a suitable grinder.

Fine oilseeds (such as linseed, rape- and sesame seeds) may be crushed. Take care that, during preparation, no change occurs in the moisture content of the sample.

Weigh the sample for analysis (m_0), to the nearest 0,1 g, in a suitable extraction thimble and cover it with fat-free cotton wool. Extract for 2 to 3 h with the diethyl ether (5.1.1) or the *n*-hexane (5.1.2) in the Soxhlet type extractor (6.2.1) with a minimum of ten syphonings per hour or at a rate of at least 5 drops per second for continuous extraction apparatus.

If extraction with a warm solvent may cause changes in the nature of the sample, cold extraction with *n*-hexane is recommended.

If the extraction is carried out with diethyl ether, remove the solvent from the flask (mass of empty flask : m_2) by distillation, and dry the contents of the flask (horizontally) in the oven (6.2.2), controlled at 103 ± 2 °C, to constant mass (i.e. until the difference between two consecutive weighings does not exceed 10 mg).

Cool in the desiccator and again weigh the flask to the nearest 1 mg (m_3).

The mass of extracted fat is equal to

$$m_3 - m_2$$

If the extraction is carried out using *n*-hexane, distil until the flask is nearly free of solvent, add 2 ml of the acetone (5.1.3) to the fat, swirl, warm gently on a suitable heating device to remove the acetone (the last traces can be removed by blowing) and continue as for diethyl ether, drying for 10 min in the oven (6.2.2), controlled at 103 ± 2 °C.

If it is not possible, because of the amount of fat present, to obtain a mass which is constant within the required limits, dissolve the extract in carbon tetrachloride (5.1.4), transfer to a 500 ml volumetric flask, dilute to the mark with the same carbon tetrachloride and mix.

Transfer 50,0 ml to a tared flask (m_2) containing a few silicon carbide chips. Proceed as for a normal extraction.

The mass of extracted fat is equal to

$$10\,(m_3 - m_2)$$

Allow the solvent to evaporate at ambient temperature and then condition the de-fatted residue for 2 h in the ambient atmosphere, preferably in a room with the same temperature and relative humidity as the room in which grinding was carried out, and weigh (m_1). Grind the air-dry residue so that it passes through a sieve of nominal aperture size 1 mm. Store the test sample in an airtight container.

The results of analyses may be referred to the original product by means of the conversion factor described in clause 9.

8.3.5 Samples having high fat and high moisture contents and which are difficult to crush

8.3.5.1 Weigh the sample for analysis (m_0), to the nearest 0,1 g. Pre-dry the sample in the vacuum oven (6.3.2), controlled at 80 ± 2 °C, weigh to the nearest 0,1 g (m_1), after conditioning in the ambient atmosphere, and carry out the extraction of fat as described in 8.3.4.

8.3.5.2 For samples which are difficult to dry, for example because of a fatty layer covering the surface of the wet material, mix with a known quantity of the diatomaceous earth (5.2) before drying. The quantity of diatomaceous earth to be added is related to the water and fat contents. Proceed as described in 8.3.5.1.

NOTE — Diatomaceous earth behaves like an inert material but is found as a part of the ash content and possibly in the mineral content.

The results of such analyses should, therefore, be corrected for the quantity of diatomaceous earth added.

9 Calculation of conversion factor

If pre-drying or pre-extraction of fat has been carried out, the results of subsequent analyses shall be referred to the original product by multiplication by the conversion factor

m_1/m_0

where

m_0 is the mass, in grams, of the portion of sample for analysis taken;

m_1 is the mass, in grams, of this portion of the sample for analysis after extraction and/or drying.

If the sample is to be used for the determination of the fat content[1], diethyl ether extract (ISO 5986) and/or moisture content (ISO 6496), the results of the pre-extraction or pre-drying shall be included in the final calculation of the respective contents.

1) A method for the determination of fat content will form the subject of ISO 6492.

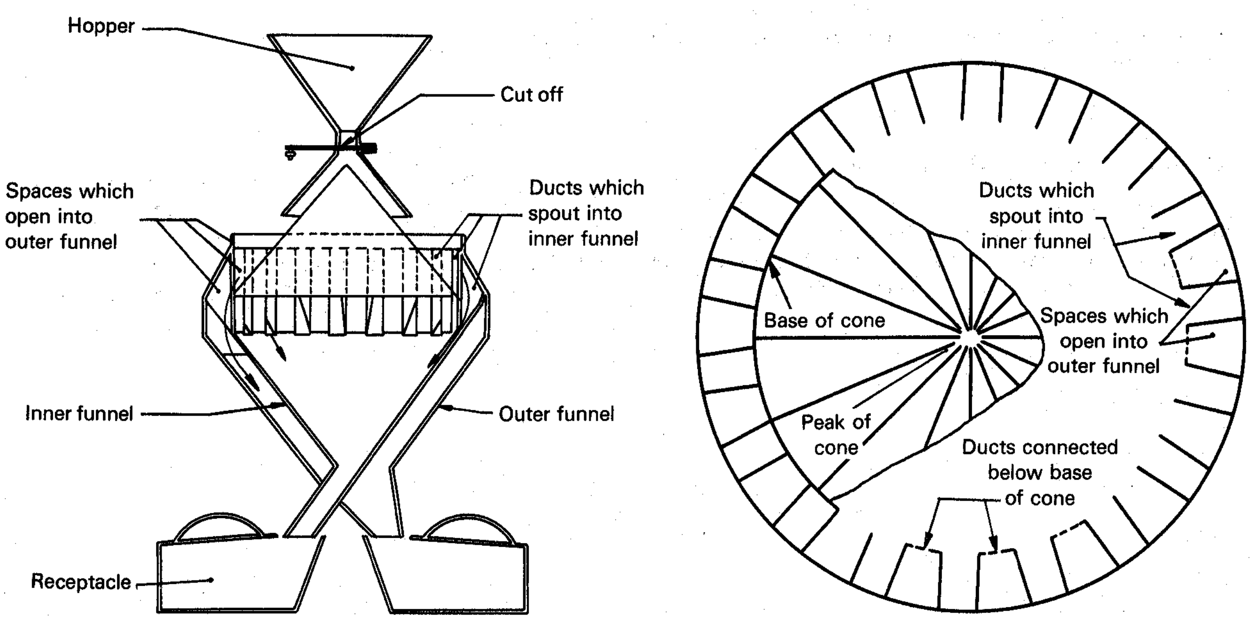

Figure 1 — Conical divider

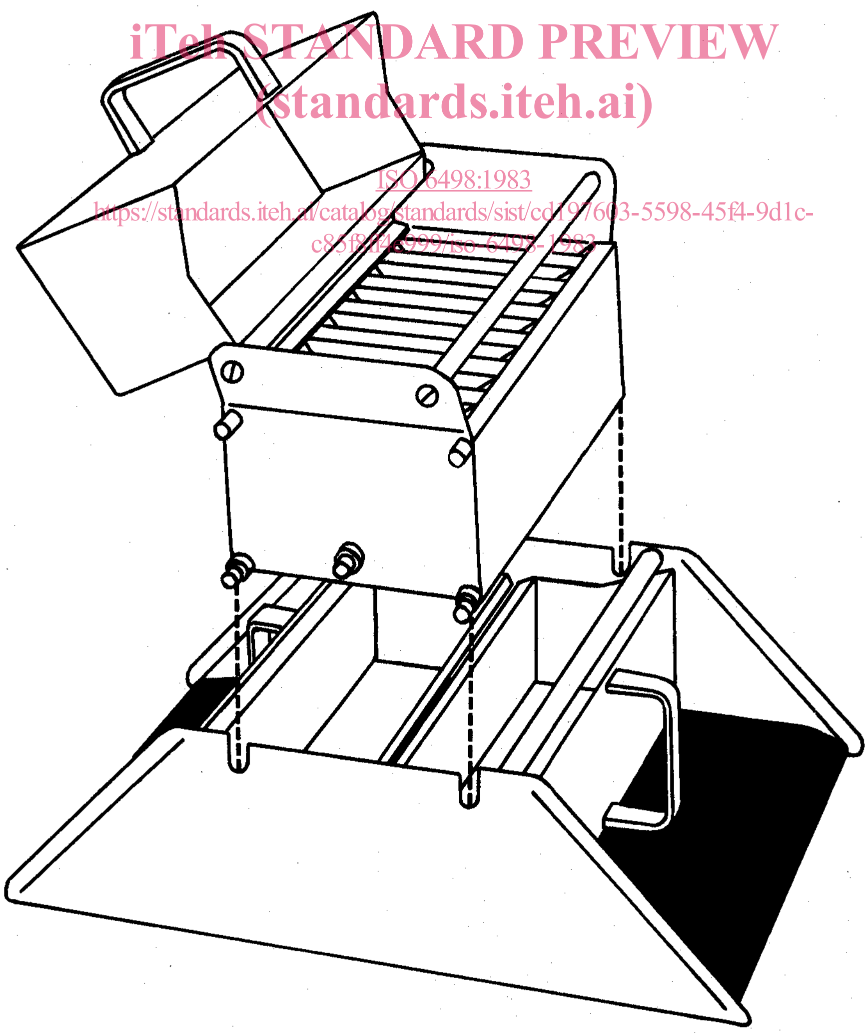

Figure 2 — Multiple-slot divider with sorting system