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Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration

Aliments des animaux — Détermination de la teneur en cellulose brute — Méthode avec filtration intermédiaire

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6865 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A of this International Standard is for information only.

Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration

1 Scope

This International Standard specifies a method with intermediate filtration for the determination of the crude fibre content. A manual procedure and a semi-automatic procedure are described.

The method is applicable to animal feeding stuffs with a crude fibre content greater than 10 g/kg.

NOTE For animal feeding stuffs with a crude fibre content equal to or less than 10 g/kg, the method described in ISO 6541 [7] may be used.

This International Standard is also applicable to cereals and pulses.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative documents referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples.*

3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

3.1
crude fibre content
loss in mass resulting from ashing of the dried residue obtained after acid and alkaline digestion of the sample by the procedure described in this International Standard, divided by the mass of the test portion

NOTE The crude fibre content is expressed in grams per kilogram. It may also be expressed as a mass fraction in percent.

4 Principle

The test portion is treated with boiling dilute sulfuric acid. The residue is separated by filtration, washed and then treated with boiling potassium hydroxide solution. The residue is separated by filtration, washed, dried, weighed and then ashed. The loss in mass resulting from ashing corresponds to the mass of crude fibre in the test portion.

5 Reagents and materials

Use only reagents of recognized analytical grade.

5.1 Water, complying with at least grade 3 in accordance with ISO 3696.

5.2 Hydrochloric acid, c(HCl) = 0,5 mol/l.

5.3 Sulfuric acid, $c(H_2SO_4)$ = (0,13 ± 0,005) mol/l.

5.4 Potassium hydroxide solution, c(KOH) = (0,23 ± 0,005) mol/l.

5.5 Acetone.

5.6 Filter aid: sea sand , or Celite®[1)] 545, or material of an equivalent quality.

Before use, treat sea sand with boiling hydrochloric acid [c(HCl) = 4 mol/l], wash with water until free from acid and heat at a temperature of (500 ± 25) °C for at least 1 h.

Before use, heat other filter aids at a temperature of (500 ± 25) °C for at least 4 h.

5.7 Antifoam agent, for instance *n*-octanol.

5.8 Light petroleum, boiling range 40 °C to 60 °C.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Grinding device, capable of grinding the sample so that it passes completely through a **sieve** with 1 mm apertures.

6.2 Analytical balance, with accuracy of at least 0,1 mg.

6.3 Filter crucibles, of quartz, porcelain or hard glass, with fused sintered filter plate with a pore size of 40 µm to 100 µm (porosity grade P 100 according to ISO 4793:1980 [1]).

Before using for the first time, carefully and gradually heat a new filter crucible to a temperature not exceeding 525 °C and leave for a few minutes at (500 ± 25) °C.

Stainless-steel crucibles with stainless-steel sieve plates with an aperture size of 90 µm of identical performance characteristics may be used as an alternative.

6.4 Porcelain sieve plates.

6.5 Incineration dishes.

6.6 Beakers or **conical flasks,** of 500 ml capacity, provided with a suitable cooling device, for example a condensor or a dish.

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1) Celite® is the tradename of a commercially available product. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

6.7 Drying oven, electrically heated and ventilated, capable of being maintained at a temperature of (130 ± 2) °C.

6.8 Desiccator, containing blue silicagel as desiccant, provided with a perforated plate, preferably of aluminium or stainless steel, of thickness 2 mm to 3 mm.

6.9 Muffle furnace, electrically heated, provided with air circulation and temperature control capable of maintaining the temperature around the crucibles to the nearest 25 °C at temperatures of 475 °C to 525 °C.

The reading of the pyrometer of the muffle furnace cannot always be trusted: deviations may occur. Therefore, the temperature in the muffle furnace should be checked regularly.

Depending on the size and type of the muffle furnace, temperatures in the furnace may differ from one place to another place. When the oven door is closed, adequate air supply should be guaranteed. The volume flow rate of air should not be so great that material is carried away from the crucibles.

6.10 Cold-extraction device, provided with

— a support for the filter crucible (6.3);

— a discharge pipe with a tap to the vacuum and liquid outlet; and

— connecting rings for connecting the filter crucible (6.3).

6.11 Heating device (for manual method), provided with a suitable cooling device capable of maintaining the volume constant during boiling.

6.12 Heating device (for the semi-automatic method) for acid and alkaline digestion, provided with

— a support for the filter crucible (6.3);

— a discharge pipe with a tap to the vacuum and liquid outlet;

— a boiling cylinder of at least 270 ml capacity, with a reflux condenser;

— connecting rings for connecting the heating device to the filter crucible (6.3) and the boiling cylinder; and

— optionally, provision for compressed air.

Before use, preheat the apparatus with boiling water for 5 min.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [6].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Using the grinding device (6.1), grind the air-dry laboratory sample so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

For the manual method, proceed in accordance with clause 9.

For the semi-automatic method, proceed in accordance with clause 10.

9 Procedure for manual method

9.1 Test portion

Weigh about 1 g of the prepared test sample (clause 8) to the nearest 0,1 mg (m_1).

If the fat content of the sample exceeds 100 g/kg, or if the sample contains fats which cannot be extracted directly with light petroleum (5.8), transfer the sample to a crucible (6.3) and proceed in accordance with 9.2.

If the fat content of the sample does not exceed 100 g/kg, transfer the sample to a beaker (6.6) and proceed in accordance with 9.3 if the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg. If this is not the case, proceed in accordance with 9.4.

9.2 Preliminary defatting

In the cold-extraction device (6.10), defat the sample three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing. Transfer the residue to a beaker (6.6).

If the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg, proceed in accordance with 9.3. If not, proceed in accordance with 9.4.

9.3 Removal of carbonate

Pour 100 ml of hydrochloric acid (5.2) over the sample. Stir continuously for 5 min. Carefully pour the mixture into a filter crucible, the bottom of which is covered with a thin layer of filter aid (5.6).

Decant twice with 100 ml of water each time. Take care so that as little material as possible ends up on the filter.

Transfer the contents of the crucible to the original beaker and proceed in accordance with 9.4.

9.4 Acid digestion

Pour 150 ml of sulfuric acid (5.3) over the sample.

Bring to the boil as quickly as possible and continue steady boiling for (30 ± 1) min.

At the beginning of boiling, swirl a few times. If foaming occurs, add a few drops of antifoam agent (5.7). During boiling, maintain a constant volume by using a suitable cooling device (see 6.6 and 6.11).

9.5 First filtration

In the filter crucible (6.3) apply a layer of filter aid (5.6) with a thickness of about one-fifth of the height of the filter crucible. The filter aid may be covered by a sieve plate (6.4) to prevent splashing.

When the boiling time has elapsed, filter the liquid down a stirring rod into the filter crucible. Apply a weak vacuum, so that just in one pass 150 ml is almost completely poured. If the filter blocks, carefully shift aside with a stirring rod the crude fibre covering the filter aid.

Wash the residue five times with about 10 ml of hot water each time. Take care that the filter plate of the crucible remains covered by the filter aid, so that the crude fibre will not reach the filter plate.

Release the vacuum and transfer a volume of acetone (5.5) which is sufficient to just cover the residue. Wait a few minutes and then remove the acetone by applying slight suction. For a few moments suck air through to dry the residue.

If the sample contains fats which cannot be extracted directly with light petroleum (5.8), proceed in accordance with 9.6. If not, proceed in accordance with 9.7.

9.6 Defatting

In the cold-extraction device (6.10), defat the sample three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing.

9.7 Alkaline digestion

Transfer the residue quantitatively into the same beaker used for the acid digestion.

Add 150 ml of potassium hydroxide solution (5.4) and bring to the boil as quickly as possible. Continue steady boiling for (30 ± 1) min. During boiling, maintain a constant volume by using a suitable cooling device (see 6.6 and 6.11).

9.8 Second filtration

Filter the contents of the beaker through the filter crucible (6.3) containing a layer of filter aid (5.6) with a thickness of about one-fifth of the height of the filter crucible, covered by a sieve plate (6.4) to prevent splashing.

Wash the residue with hot water until the rinsings are neutral.

Wash the residue three times under vacuum with 30 ml of acetone (5.5) each time. Dry the residue by suction after each washing.

9.9 Drying

Place the filter crucible in an incineration dish (6.5) and dry the dish with its contents for at least 2 h in the drying oven (6.7) set at a temperature of 130 °C.

During ashing or cooling, parts of the sintered filter plate of the crucible may come loose. As this may cause an incorrect analysis result, place the filter crucible in an incineration dish.

Leave the filter crucible and the incineration dish to cool in the desiccator (6.8). Immediately after removal from the desiccator, weigh the filter crucible and the incineration dish to the nearest 0,1 mg (m_2).

9.10 Ashing

Place the filter crucible and the incineration dish in the muffle furnace (6.9) and ash its contents at a temperature of (500 ± 25) °C until the difference between two consecutive weighings after cooling does not exceed 2 mg.

After each ashing, leave the filter crucible and the incineration dish to cool partly and, while still warm, place in the desiccator. Leave to cool completely then weigh to the nearest 0,1 mg (m_3).

9.11 Blank determination

Carry out a blank determination as described in 9.4 to 9.10 with about the same quantity of filter aid (5.6) but without the sample.

The loss in mass resulting from ashing (9.10) shall not exceed 2 mg.

Proceed in accordance with clause 11.