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Animal feeding stuffs — Determination of fat content

Aliments des animaux — Détermination de la teneur en matière grasse

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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.

International Standard ISO 6492 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A of this International Standard is for information only.

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Animal feeding stuffs — Determination of fat content

1 Scope

This International Standard specifies a method for the determination of the fat content of animal feeding stuffs. The method is applicable to animal feeding stuffs except oilseeds and oilseed residues.

For the purposes of this method, the following two categories of animal feeding stuffs are distinguished. Samples of products in category B need a hydrolysis step prior to extraction.

Category B:

— straight feeds of animal origin including milk products;

— straight feeds of vegetable origin from which fats cannot be extracted without prior hydrolysis; in particular: gluten, yeast, soya and potato proteins, and heat-treated feeds;

— compound feeds containing the preceding products in such quantities that at least 20 % of the fat content stems from these products.

Category A:

— animal feeding stuffs not mentioned under category B.

NOTE For oilseed residues, a method for the determination of the "oil content" by hexane extraction is specified in ISO 734-1 [2], whereas a method for the determination of the "oil content" by diethyl ether extraction is specified in ISO 736 [3].

For oilseeds, a method for the determination of the "oil content" by hexane extraction is specified in ISO 659 [1].

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 document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 6498, *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
fat content
mass fraction of substances extracted from the sample by the procedure specified in this International Standard

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

4 Principle

4.1 Samples with a relative high fat content (at least 200 g/kg) undergo a preliminary extraction with light petroleum.

4.2 For samples of category B, the sample is hydrolysed with hydrochloric acid under heating. The solution is cooled and filtered. The residue is washed and dried then extracted with light petroleum. The solvent is removed by distillation and drying. The residue is weighed.

4.3 For samples of category A, the sample is extracted with light petroleum. The solvent is removed by distillation and drying. The residue is weighed.

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 Sodium sulfate, anhydrous.

5.3 Light petroleum, consisting mainly of hydrocarbons with six carbon atoms, boiling range 40 °C to 60 °C.

The bromine value shall be less than 1. The evaporation residue shall be less than 20 mg/l.

Alternatively, technical hexane may be used having an evaporation residue of less than 20 mg/l.

5.4 Silicon carbide chips or **glass beads**.

5.5 Acetone.

5.6 Hydrochloric acid, c(HCl) = 3 mol/l.

5.7 Filtration aid, for example diatomaceous earth (Kieselguhr), boiled for 30 min in hydrochloric acid, c(HCl) = 6 mol/l, washed with water until acid-free, then dried at 130 °C.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Extraction thimbles, free from fats and oils, ether-washed.

6.2 Soxhlet-type extractor, with syphoning volume of about 100 ml, or other recirculation extractor.

6.3 Heating apparatus, with temperature control, not liable to act as an ignition source.

6.4 Drying oven, capable of being maintained at (103 ± 2) °C.

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

6.6 Desiccator, containing an efficient desiccant.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [4].

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

Store the sample in such a way that deterioration and change in composition are minimized.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

9 Procedure

9.1 Procedure selection

If the test sample is difficult to crush, or if it is difficult to obtain a homogeneous reduced test sample because of a high fat content (exceeding 200 g/kg), proceed in accordance with 9.2.

In all other cases proceed in accordance with 9.3.

9.2 Preliminary extraction

9.2.1 Weigh (m_0) at least 20 g of the prepared test sample (clause 8) to the nearest 1 mg and mix with 10 g of anhydrous sodium sulfate (5.2). Transfer to an extraction thimble (6.1) and cover with a fat-free wad of cottonwool.

Transfer some silicon carbide chips (5.4) to a dry flask. If the fat has to undergo subsequent quality tests, use glass beads instead of silicon carbide chips. Connect the flask with the extractor to collect the light petroleum extract.

Place the thimble in the extractor (6.2) and extract for 2 h with light petroleum (5.3). Regulate the heating apparatus (6.3) to obtain at least 10 siphonings per hour if a Soxhlet-type extractor is used, or a reflux rate of at least 5 drops per second (about 10 ml/min) if an equivalent apparatus is used.

Dilute the light petroleum extract in the flask to 500 ml with light petroleum (5.3) and mix well. Weigh (m_1), to the nearest 1 mg, a dry flask containing some silicon carbide chips or glass beads (5.4). Pipette 50 ml of the light petroleum solution into this flask.

9.2.2 Distil off the solvent until the flask is nearly free from solvent. Add 2 ml of acetone (5.5) to the flask, swirl and gently warm on the heating apparatus (6.3) to remove the acetone. Blow off the last traces of acetone. Dry the residue for (10 ± 0,1) min in the drying oven (6.4) set at 103 °C. Cool in the desiccator (6.6) and weigh (m_2) to the nearest 0,1 mg.

Alternatively, the following procedure may be applied.

Distil off the solvent. Dry the residue in the flask for 1,5 h under vacuum in the oven (6.5) set at 80 °C. Leave to cool in the desiccator (6.6) and weigh (m_2) to the nearest 0,1 mg.

9.2.3 Leave the extraction residue from the thimble to dry in air to eliminate solvent residues. Weigh (m_3) the dried residue to the nearest 0,1 mg.

Crush the residue to a particle size of 1 mm.

Proceed in accordance with 9.3.

9.3 Test portion

Weigh (m_4), to the nearest 1 mg, 5 g of the prepared test sample (clause 8 or 9.2).

For a sample of category B (see clause 1), proceed in accordance with 9.4.

For a sample of category A, transfer the test portion to an extraction thimble (6.1) and cover with a fat-free wad of cottonwool. Proceed in accordance with 9.5.

9.4 Hydrolysis

Transfer the test portion to a 400 ml beaker or a 300 ml conical flask. Add 100 ml of hydrochloric acid (5.6) and silicon carbide chips (5.4). Cover the beaker with a watch glass or fit the conical flask with a reflux condensor. Bring the mixture to a gentle boil over a flame or a hot plate and maintain it for 1 h. Swirl every 10 min to prevent the product sticking to the sides of the container.

Cool to ambient temperature and add a quantity of filtration aid (5.7) sufficient to prevent any loss of fat during the filtration. Filter through a moistened, fat-free double filter paper in a Büchner funnel with suction. Wash the residue with cold water until a neutral filtrate is obtained.

CAUTION: If oil or fat appears on the surface of the filtrate, wrong results may be obtained. A possible solution is to repeat the procedure applying a smaller test portion or a higher acid concentration.

Carefully take out the filter and place the double filter paper containing the residue in an extraction thimble (6.1) and dry under vacuum for 60 min in the oven (6.5) set at 80 °C. Remove the thimble from the oven and cover with a fat-free wad of cottonwool.

9.5 Extraction

9.5.1 Transfer some silicon carbide chips (5.4) to a dry flask and weigh (m_5) to the nearest 1 mg. If the fat has to undergo subsequent quality tests, use glass beads instead of silicon carbide chips. Connect the flask with the extractor to collect the light petroleum extract.

Place the thimble in the extractor (6.2) and extract for 6 h with light petroleum (5.3). Regulate the heating apparatus (6.3) to obtain at least 10 siphonings per hour if a Soxhlet-type extractor is used, or a reflux rate of at least 5 drops per second (about 10 ml/min) if an equivalent apparatus is used.

9.5.2 Distil off the solvent until the flask is nearly free from solvent. Add 2 ml of acetone (5.5) to the flask, swirl and gently warm on the heating apparatus (6.3) to remove the acetone. Blow off the last traces of acetone. Dry the residue for (10 ± 0,1) min in the drying oven (6.4) set at 103 °C. Cool in the desiccator (6.6) and weigh (m_6) to the nearest 0,1 mg.

Alternatively, the following procedure may be applied.

Distil off the solvent. Dry the residue in the flask for 1,5 h under vacuum in the oven (6.5) set 80 °C. Leave to cool in the desiccator (6.6) and weigh (m_6) to the nearest 0,1 mg.

10 Calculation

10.1 Determination with preliminary extraction (9.2)

Calculate the fat content of the test sample, w_1, in grams per kilogram, using the equation:

$$w_1 = \left[\frac{10\,(m_2 - m_1)}{m_0} + \left(\frac{(m_6 - m_5)}{m_4} \times \frac{m_3}{m_0}\right)\right] \times f$$

where

m_0 is the mass of the test sample weighed in 9.2, in grams (g);

m_1 is the mass of the flask with silicon carbide chips used in 9.2, in grams (g);

m_2 is the mass of the flask with silicon carbide chips and the dried light petroleum extract residue obtained in 9.2, in grams (g);

m_3 is the mass of the dried extraction residue obtained in 9.2, in grams (g);

m_4 is the mass of the test portion (9.3), in grams (g);

m_5 is the mass of the flask with silicon carbide chips used in 9.5, in grams (g);.

m_6 is the mass of the flask with silicon carbide chips and dried light petroleum extract residue obtained in 9.5, in grams (g);

f is the units correction factor, in grams per kilogram (f = 1000 g/kg).

Express the result to the nearest 1 g/kg.

10.2 Determination without preliminary extraction

Calculate the fat content of the test sample, w_2, in grams per kilogram, using the equation:

$$w_2 = \frac{(m_6 - m_5)}{m_4} \times f$$

where

m_4 is the mass of the test portion (9.3), in grams (g);

m_5 is the mass of the flask with silicon carbide chips used in 9.5, in grams (g);

m_6 is the mass of the flask with silicon carbide chips and dried light petroleum extract residue obtained in 9.5, in grams (g);

f is the units correction factor, in grams per kilogram (f = 1000 g/kg).

Express the result to the nearest 1 g/kg.

11 Precision

11.1 Interlaboratory test

Details of the interlaboratory tests on the precision of the method are given in annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit r given in or derived from Table 1.

Table 1 — Repeatability limit (r) and reproducibility limit (R)

Sample	r g/kg	R g/kg
Category B (hydrolysis necessary)	5,0	12,0 [a]
Category A (hydrolysis not necessary)	2,5	7,7 [b]
[a] Except for fish meal and meat meal; see Table A.1. [b] Except for coconut meal; see Table A.2.		

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit R given in or derived from Table 1.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A
(informative)

Results of interlaboratory tests

The precision of the method was established by three interlaboratory tests organized by the member bodies of the former Czechoslovakia, the Netherlands and France in 1984 and carried out in accordance with ISO 5725 [5][1)].

In the test in the former Czechoslovakia, 21 laboratories participated. Samples of dried whole milk, fish meal, mixed broiler feed and pelleted cattle feed were investigated.

In the test in France, 33 laboratories participated. Samples of brewery solubles, maize, meat meal, soya bean meal and turkey feed were investigated.

In the test in the Netherlands, 10 laboratories participated. Samples of barley, bone meal, coconut meal, feather meal, maize gluten feed and two mixed feeds were investigated .

See Tables A.1 and A.2 for a summary of the statistical results of the tests.

NOTE More detailed information is given in document ISO/TC 34/SC 10 N 353.

Table A.1 — Statistical results of interlaboratory tests on samples of category B (hydrolysis necessary)

Parameter	Sample [a]										
	1	**2** [b]	**3**	**4**	**5**	**6**	**7**	**8** [b]	**9** [b]	**10**	**11**
Number of laboratories retained after eliminating outliers	25	8	23	24	18	23	27	8	8	19	20
Mean fat content, g/kg [c]	23,4	40,2	44,7	55,0	78,0	80,9	88,4	101,3	150,6	188,0	251,0
Repeatability standard deviation, s_r, g/kg	—	0,78	—	—	1,73	—	—	1,20	1,73	1,24	1,84
Repeatability coefficient of variation, %	—	1,98	—	—	2,23	—	—	1,20	1,13	0,67	0,74
Repeatability limit, r $(2{,}8 \times s_r)$, g/kg	—	2,2	—	—	4,9	—	—	3,4	4,9	3,5	5,2
Reproducibility standard deviation, s_R, g/kg	4,03	1,98	2,57	4,38	5,55	2,97	5,69	1,80	3,32	2,65	3,39
Reproducibility coefficient of variation, %	17,29	4,95	5,77	7,98	7,10	3,68	6,44	1,77	2,23	1,41	1,34
Reproducibility limit, R $(2{,}8 \times s_R)$, g/kg	11,4	5,6	7,3	12,4	15,7	8,4	16,1	5,1	9,4	7,5	9,6

[a] Sample 1: soya bean meal;
Sample 2: maize gluten feed;
Sample 3: maize;
Sample 4: brewery solubles;
Sample 5: fish meal;
Sample 6: turkey feed;
Sample 7: meat meal;
Sample 8: feather meal;
Sample 9: bone meal;
Sample 10: pelleted cattle feed;
Sample 11: dried whole milk.

[b] Results expressed based on dry matter.

[c] Applied solvent: light petroleum, boiling range 40 °C to 60 °C.

1) ISO 5725:1986 (now withdrawn) was used to obtain the precision data.