

INTERNATIONAL STANDARD

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Animal feeding stuffs — Determination of soluble nitrogen content after treatment with pepsin in dilute hydrochloric acid

*Aliments des animaux — Détermination de la teneur en azote soluble après
traitement avec de la pepsine dans l'acide chlorhydrique dilué*



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Foreword

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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 6655 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A forms an integral part of this International Standard.

Annexes B and C are for information only.

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Animal feeding stuffs — Determination of soluble nitrogen content after treatment with pepsin in dilute hydrochloric acid

1 Scope

This International Standard specifies a method for the determination of the soluble nitrogen content of animal feeding stuffs after treatment with pepsin in dilute hydrochloric acid.

This method does not distinguish between protein nitrogen and non-protein nitrogen.

NOTES

1 The values obtained on using this method have no direct connection with digestibility *in vivo*.

2 If non-protein nitrogen is to be excluded from the test result, its content should be determined using an appropriate method.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5983: 1997¹, *Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content — Kjeldahl method*.

ISO 6498:1983, *Animal feeding stuffs - Preparation of test samples*.

3 Principle

Incubation of the sample for 48 h at 40 °C in a solution of pepsin in dilute hydrochloric acid.

Filtration of the suspension and determination of the nitrogen content of either the filtrate or the residue remaining in the filter in accordance with the Kjeldahl method specified in ISO 5983. In the latter case, determination of the nitrogen content of the sample as well, in accordance with ISO 5983.

4 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of at least equivalent purity.

The reagents (except the standard materials) shall be practically free from nitrogenous compounds.

¹ To be published. (Revision of ISO 5983:1979)

4.1 Dilute hydrochloric acid, $c(\text{HCl}) = 0,075 \text{ mol/l}$.

4.2 Pepsin, having an activity of 2,0 units per milligram in accordance with the definition given in annex A. Check the pepsin activity in accordance with the method specified in annex A.

4.3 Pepsin solution in hydrochloric acid, with a pepsin activity of about 400 units per litre.

Dissolve $0,2 \text{ g} \pm 0,001 \text{ g}$ of pepsin (4.2) in 1 l of dilute hydrochloric acid (4.1). Prepare this solution immediately before use.

If the activity of the pepsin deviates from 2,0 units per milligram, adjust the mass of pepsin to obtain a solution with a pepsin activity of 400 units per litre.

4.4 Hydrochloric acid, $c(\text{HCl}) = 7,5 \text{ mol/l}$ ($\rho_{20} = 1,125 \text{ g/ml}$).

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Water bath or incubator, capable of being maintained at $40 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$.

5.2 Kjeldahl digestion flasks, of suitable capacity.

5.3 Filter paper, fast filtration rate, acid-resistant.

5.4 Distillation and titration equipment

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [3].

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

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

If the fat content of the test sample exceeds 10 % (m/m), extract the fat in accordance with ISO 6498, and take the content of extracted fat into account in the calculation (clause 9).

8 Procedure

8.1 Test portion

Weigh, to the nearest 0,001 g, about 2 g of the prepared test sample.

8.2 Incubation

Place the test portion in a 500 ml volumetric flask and add 450 ml of the pepsin solution (4.3) previously heated to $40 \text{ }^{\circ}\text{C}$. Shake so as to avoid agglomeration. Check that the pH of the suspension is lower than 1,7. Place the flask in the water bath or incubator (5.1) set at $40 \text{ }^{\circ}\text{C}$ and leave it there for 48 h. Shake after 8 h, 24 h and 32 h.