INTERNATIONAL STANDARD

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Oilseed meals — Determination of soluble proteins in potassium hydroxide solution

Tourteaux de graines oléagineuses — Détermination de la teneur en protéines solubles en solution d'hydroxyde de potassium

Reference number
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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 2, *Oleaginous seeds and fruits and oilseed meals*.

Oilseed meals — Determination of soluble proteins in potassium hydroxide solution

1 Scope

This International Standard specifies a method for the determination of soluble proteins in potassium hydroxide solution in soya meals, rapeseed meals and sunflower pellets, which are then assayed using the Kjeldahl method as specified in ISO 5983-1 and ISO 5983-2.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

ISO 5500, *Oilseed residues — Sampling*

ISO 5502, *Oilseed residues — Preparation of test samples*

ISO 5983-1, *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 1: Kjeldahl method*

ISO 5983-2, *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 2: Block digestion and steam distillation method*

3 Principle

The sample is dispersed in a solution of potassium hydroxide of approximately 12,5 pH, stirred and centrifuged. Then, the nitrogen content of the clarified liquid is determined by the Kjeldahl method for crude protein and compared with the value of crude protein of the original sample.

NOTE The Kjeldahl method is described in ISO 5983-1 and ISO 5983-2.

4 Reagents

WARNING 1 The tests, according to this International Standard, involve risks for persons and the possibility of releasing substances which might cause damage to the environment. For this reason, appropriate measures shall be taken to prevent risk, protect personnel, and avoid the release of the substances involved.

WARNING 2 Attention shall be paid to preserving the environment in all phases of this activity. For further information, it is recommended to make reference to ASTM D4447, which describes the classification of the kind of residues and pretreatment methods for their recovery or disposal.

Use only reagents of recognized analytical grade.

4.1 Potassium hydroxide.

4.2 Potassium hydroxide solution, c(KOH) = 0,036 mol/l.

Preparation: Dissolve 2,4 g of potassium hydroxide (mass fraction w = 85 g/100 g) in 1 000 ml of distilled water.

4.3 n-hexane or hexane mixed isomers or petroleum ether.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Sieve, 500 µm for sunflower pellets and 250 µm for soya and rapeseed meals (as specified in ISO 565).

5.2 Analytical balance, capable of weighting to the nearest 0,001 g.

5.3 Stirrer's vessels, of 150 ml capacity.

5.4 Magnetic stirrer with revolutions per minute (r/min) indicator or mechanical rotary stirrer, composed of an axis, and allowing the centrifuge tubes to invert totally as the axis rotates.

5.5 Grinder.

5.5.1 Cutting mill, type of coffee grinder or grinder equipped with a grid or equivalent.

5.5.2 Cyclone mill, or similar.

5.6 Centrifuge, allowing reaching a relative acceleration of 800 g ± 100 g.

The value of the rotational frequency, *v*, is calculated using Formula (1):

$$v = 423\sqrt{\frac{F_c}{d}} \quad (1)$$

where

v is the rotational frequency, in revolutions per minute;

d is the spinning diameter, in centimetres, measured between the ends of the opposite tubes, in rotation position;

F_c is the relative centrifugal acceleration (in this case, 800 g).

5.7 One-mark volumetric pipettes, of 25 ml capacity.

5.8 Burette, of 100 ml capacity.

5.9 Centrifuge tube or centrifugation ampoule.

5.10 Filter paper, nitrogen free or glass pot, with a filter plate.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5500.

It is important that the laboratory receives 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 5502.

If the fat content of the sample is higher than 5 %, it shall be defatted by cold extraction using n-hexane.

8 Procedure

8.1 Carry out the determination in duplicate.

8.2 Grind the sample with a grinder or with any other apparatus which does not cause warming until it totally passes through a 250 µm sieve for soya and rapeseed meals and through a 500 µm sieve for sunflower pellets.

Particle size greatly affects the final result of the analysis; therefore, it is recommended to carry out the milling with care.

8.3 Weigh 1,5 g of the meal prepared as in 8.2, and place it in a 150 ml stirrer's vessel. If a mechanical rotator stirrer is employed, the stirrer's recipient should be used instead of the stirrer's vessel.

8.4 Add 75 ml (5.8) of potassium hydroxide solution (4.2) and stir at minimum speed for 20 min to maintain all the solids in suspension. If a stirrer is utilized, use the centrifuge tube with the stirring sets at minimum speed.

8.5 Transfer the totality of the liquid to a centrifugal tube or centrifugation ampoule and centrifuge for 10 min at a relative acceleration of 800 g.

8.6 If some particles are still in suspension, filter the clarified liquid through filter paper or a glass pot to prevent the possible transfer of particles.

8.7 Take 25 ml (5.7) aliquots of filtrate and determine the nitrogen content by the Kjeldhal method as described in ISO 5983-1 or ISO 5983-2.

NOTE According to this procedure, each aliquot corresponds to 0,5 g of the original milled sample.

8.8 The nitrogen content of the original milled sample shall be determined in duplicate using the Kjeldahl method as described in ISO 5983-1 or ISO 5983-2.

9 Expression of results

The content of soluble proteins in potassium hydroxide solution, w_{sp}, expressed as a mass fraction, in grams of soluble proteins (from supernatant) per 100 g of the total protein, is calculated using Formula (2):

$$w_{sp} = \frac{N_s}{N_t} \times 100 \qquad (2)$$

where

w_{sp} is the soluble proteins content in potassium hydroxide solution, in grams per 100 g;

N_s is the nitrogen content obtained as in 8.7;

N_t is the nitrogen content obtained as in 8.8.

Report the results to one decimal place.

10 Precision

10.1 Interlaboratory test

The results of an interlaboratory test are given in Annex A for information.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test materials in the same laboratory by the same operator using the same equipment within a short interval of time, will not exceed the arithmetic mean of the values for *r* obtained from the interlaboratory study in more than 5 % of cases given in Table A.1.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test materials in different laboratories with different operators using different equipment will not exceed the arithmetic mean of the values for *R* obtained from the interlaboratory study in more than 5 % of cases given in Table A.1.

11 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, with reference to this International Standard, i.e. ISO 14244;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which might have influenced the results;

— the test results obtained;

— if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory tests

An interlaboratory test was carried out by the Argentinean Certification and Standardization Institute (IRAM) in order to evaluate the repeatability and reproducibility of the test method in this International Standard.

Ten test samples of three different matrixes (soybean meal, rapeseed meal and sunflower pellets) were sent to 24 national and foreign laboratories, and 21 laboratories results were received. To sum up, 87,5 % of laboratories participated actively in this interlaboratory test.

The calculation of repeatability and reproducibility values obtained for soluble proteins arises from the application of a statistical analysis according to ISO 5725-1[1] and ISO 5725-2.[2] This analysis was prepared by Complejo Laboratorios of Bolsa de Comercio de Rosario (Argentina). The statistical results are shown in Table A.1.

Table A.1 — Results of the interlaboratory test

Parameter	Soybean meal 1	Soybean meal 2	Soybean meal 3	Sunflower pellets	Rapeseed meal
Number of laboratories retained after eliminating outliers	19	18	18	16	17
Overall mean, g/100 g	82,24	75,29	51,54	66,27	38,50
Standard deviation of repeatability, S_r, g/100 g	1,21	0,62	1,34	2,03	1,37
Repeatability limit, r (= 2,8 S_r)	3,38	1,73	3,75	5,68	3,82
Coefficient of variation of repeatability, $C_{V,r}$ (%)	1,47	0,82	2,60	3,06	3,55
Standard deviation of reproducibility, S_R, g/100 g	3,04	2,77	4,19	4,60	4,47
Reproducibility limit, R (= 2,8 S_R)	8,51	7,75	11,73	12,87	12,52
Coefficient of variation of reproducibility, $C_{V,R}$ (%)	3,69	3,68	8,13	6,94	11,62