# INTERNATIONAL STANDARD ISO 3099

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## Oilseed residues — Determination of total nitrogen content

*Tourteaux de graines oléagineuses — Dosage de l'azote total*

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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 3099 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products,* and circulated to the Member Bodies in April 1973.

It has been approved by the Member Bodies of the following countries :

| | | |
|---|---|---|
| Austria | France | New Zealand |
| Brazil | Germany | Poland |
| Bulgaria | Hungary | Romania |
| Canada | India | South Africa, Rep. of |
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The Member Body of the following country expressed disapproval of the document on technical grounds :

United Kingdom





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# Oilseed residues — Determination of total nitrogen content

## 0 INTRODUCTION

It is recognized that a very great number of variants of the Kjeldahl method are used in different laboratories throughout the world and that it is unlikely that one single procedure will be acceptable to all analysts.

For this reason a general text on the Kjeldahl method has already been published, namely ISO/R 1871, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method.*

This International Standard is based on the text of ISO/R 1871 and indicates the way in which the method is applicable in the particular case of oilseed residues.

## 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for the determination of the total nitrogen content of the residue remaining after treatment of oilseeds by pressure or solvent extraction.

The method is not applicable to compound products obtained from oilseed residues.

## 2 REFERENCES

ISO/R 771, *Oilseed residues — Determination of moisture and volatile matter.*

ISO/R 1871, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method.*

ISO . . ., *Oilseed residues — Sampling.* [1)]

ISO . . ., *Oilseed residues — Reduction of laboratory samples to samples for analysis.* [1)]

## 3 PRINCIPLE

Destruction of the oil cake by the Kjeldahl method, by sulphuric acid in the presence of a catalyst, rendering of the reaction products alkaline, distillation and titration of the ammonia liberated.

## 4 REAGENTS

**4.1 Sulphuric acid,** practically free from nitrogenous compounds, $\rho_{20}$ 1,83 to 1,84 g/ml.

**4.2 Catalyst,** or **"compound catalyst"** comprising :

**4.2.1 Potassium sulphate,** pure, or pure anhydrous **sodium sulphate.**

**4.2.2 Catalyst proper** (for example mercury(II) oxide, copper(II) sulphate) or a **mixture of catalysts.**

All catalysts which are effective and satisfy the blank tests and check tests described in ISO/R 1871 are acceptable.

**4.3 Sodium hydroxide** solution, practically free from carbonate, $\rho_{20}$ 1,33 g/ml (approximately 10 N or 30 % *m/m*).

**4.4 Hydrochloric acid,** standard volumetric solution, preferably 0,1 N or 0,2 N (see also 9.2).

**4.5 Titration indicator.**

Use an indicator with an end-point in the neighbourhood of pH 5,5 (for example mixed indicator prepared by adding 15 ml of a 0,1 % aqueous methylene blue solution to 100 ml of a 0,02 % solution of methyl red in 60 % methanol), or any electrometric device giving satisfactory results in the check tests indicated in ISO/R 1871.

## 5 APPARATUS

**5.1 Analytical balance.**

**5.2 Digestion, distillation and titration apparatus.**

All apparatus which satisfies the check tests described in ISO/R 1871 may be used.

## 6 SAMPLING

See ISO . . ., *Oilseed residues — Sampling.*

1) In preparation.

## 7 PROCEDURE

### 7.1 Preparation of test sample

Prepare the test sample by the method described in ISO . . ., *Oilseed residues — Reduction of laboratory samples to samples for analysis.*

### 7.2 Test portion

Without delay after the final grinding, weigh, to the nearest 0,001 g, about 2 g of the test sample (7.1). The test portion shall be representative of the laboratory sample.

### 7.3 Determination

Use any of the numerous variations of the Kjeldahl method, providing that the one selected has been carefully checked in advance and gives satisfactory results by the check tests indicated in ISO/R 1871.

Carry out the operations corresponding to the different phases of the determination (destruction, rendering alkaline, distillation and titration), taking into account the general directions given in ISO/R 1871 (see also clause 9).

Carry out two determinations on the same test sample (7.1).

## 8 EXPRESSION OF RESULTS

### 8.1 Method of calculation and formulae

**8.1.1** When the reaction products have been rendered alkaline and the distillation of the ammonia has been carried out on the whole of the acid liquor containing the products of digestion of the test portion (7.2), the total nitrogen content, as a percentage by mass, of the test sample (7.1) is equal to :

$$\frac{(V_1 - V_0) \times T \times 0{,}014}{m} \times 100$$

where

$V_1$ is the volume, in millilitres, of acid solution of known strength (4.4), required to neutralize the ammonia originating from the test portion and the reagents;

$V_0$ is the volume, in millilitres, of the same acid solution, used for the blank test described in ISO/R 1871;

$T$ is the normality of the acid solution (4.4) used for the above titrations;

$m$ is the mass, in grams, of the test portion (7.2).

Take as the result the arithmetic mean of the two determinations carried out.

Express the result to the second decimal place.

**8.1.2** When the reaction products have been rendered alkaline and the distillation of the ammonia has been carried out on an aliquot portion (see 9.2) of the acid liquor containing the digestion products of the test portion (7.2), the total nitrogen content, as a percentage by mass, of the test sample is equal to :

$$\frac{(v_1 - v_0) \times T \times 0{,}014}{m} \times \frac{V_3}{V_2} \times 100$$

where

$v_1$ is the volume, in millilitres, of acid solution of known strength (4.4), required to neutralize the ammonia originating from the aliquot portion used and from the corresponding reagents;

$v_0$ is the volume, in millilitres, of the same acid solution, used for the blank test described in ISO/R 1871;

$T$ is the normality of the acid solution (4.4) used for the above titrations;

$V_2$ is the volume, in millilitres, of the aliquot portion used;

$V_3$ is the total volume, in millilitres, of the acid liquor obtained from the test portion (7.2);

$m$ is the mass, in grams, of the test portion (7.2).

Take as the result the arithmetic mean of the two determinations carried out.

Express the result to the second decimal place.

**8.1.3** In the case of products of high moisture and volatile matter content, for which the preparation of the test sample (7.1) has been preceded by partial drying, the total nitrogen content, as a percentage by mass, of the initial product as such, is calculated by multiplying the result obtained above (see 8.1.1 or 8.1.2) by a correction coefficient, calculated according to the formula :

$$\frac{100 - U_0}{100 - U}$$

where

$U_0$ is the content of water and volatile matter, as a percentage by mass, of the sample before the partial preliminary drying, determined by the method described in ISO/R 771;

$U$ is the content of water and volatile matter, as a percentage by mass, of the partially dried test sample (7.1), determined by the method described in ISO/R 771.

**8.1.4** If required, the total nitrogen content, as a percentage by mass, can be related to the dry material and calculated according to the formula :

$$N \times \frac{100}{100 - U}$$

where

*N* is the total nitrogen content, as a percentage by mass, of the test sample (7.1), calculated as in 8.1.1 or 8.1.2;

*U* is the content of water and volatile matter of the same sample, determined by the method described in ISO/R 771.

**8.1.5** The result can also be expressed as a percentage by mass of crude protein in the product analysed, by multiplying by a conventional factor of 6,25 the total nitrogen content calculated as in 8.1.1, 8.1.2 or 8.1.3, as appropriate (if it is required to indicate the crude protein content of the product as such, or calculated as in 8.1.4 if it is a matter of relating the crude protein content to the dry matter.

Express the result thus calculated to the first decimal place only.

### 8.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0,08 g of nitrogen (corresponding to 0,5 g of crude protein) per 100 g of product.

If the difference is greater, repeat the analysis on two other test portions. If, this time, the difference still exceeds 0,08 g of nitrogen (or 0,5 g of crude protein), take as the result the arithmetic mean of the four determinations carried out.

## 9 NOTES ON THE REAGENTS AND THE PROCEDURE

**9.1** According to the nature of the catalyst and the distillation and titration procedures selected, other reagents may also be used, depending on the requirements of the procedure adopted, for example :

**9.1.1 Sodium or potassium hypophosphite** (to precipitate the mercury, when the catalyst contains this element).

**9.1.2 Boric acid**, pure, solution, 4 % or less if necessary (to collect the distilled ammonia). Prepare this solution with distilled water, freed from carbon dioxide by boiling, and store it in a flask made of glass which will not release alkali.

**9.1.3 Sodium hydroxide,** standard volumetric solution, preferably 0,1 N, free from carbonate (if the ammonia is collected in an excess of hydrochloric acid (4.4) and back titration is used).

**9.2** For products very rich in total nitrogen, it is preferable to digest a test portion of 2 g and

a) use for the titration hydrochloric acid of greater concentration than 0,1 N (for example 0,2 N or even 0,5 N), or

b) render alkaline and distil only an aliquot portion of the acid liquor containing the digestion products, previously transferred to a volumetric flask and brought to a defined volume (for example 250 ml); the quantity of this aliquot portion should be fixed in accordance with the presumed total nitrogen content of the product analysed and the normality of the acid titration solution (for example, if this is decinormal, take an aliquot portion containing not more than 50 mg of nitrogen, which corresponds to about 36 ml of 0,1 N solution).

However, the mass of the test portion may be reduced to 1 g or even to 0,5 g, but only if the test sample (7.1) is sufficiently homogeneous, which is not generally found in the case of residues.

**9.3** Start the digestion by moderate warming, in order to avoid excessive formation of foam. Increase the heat as soon as the reaction mixture has ceased foaming. For this second phase of the digestion it is advantageous to use a source of heat capable of bringing 250 ml of water at 20 °C in a 500 ml Kjeldahl flask to the boil in 4 to 5 min.

**9.4** If the determination is being carried out on a product for which the rate of digestion is unknown, it is recommended that some advance tests should be made in order to determine a suitable digestion time. For example, heat the flask, after the contents have become clarified and have ceased to change colour, for a further 30 to 90 min and compare the respective results. Additional heating of 30 to 40 min is generally sufficient.

**9.5** When the catalyst used contains mercury, this must be precipitated before the ammonia is distilled. For this purpose use, for preference, sodium or potassium hypophosphite (9.1.1) (1 g of this reagent is sufficient to precipitate up to 1 g of mercury), adding this in the dry state, after dilution of the digestion products and before rendering the medium alkaline.

**9.6** To avoid bumping during distillation, do not use zinc granules if the catalyst contains copper, as too vigorous a liberation of hydrogen may cause the dispersion of the alkaline liquid into very fine droplets which are liable to be entrained in the distillate. In place of the zinc granules, use a few glass marbles.

**9.7** If the distillate containing the ammonia is collected in a solution of boric acid (9.1.2), it is advisable to use at least 10 ml of an approximately 4 % solution in order to collect the ammonia corresponding to 20 mg of nitrogen. In this case, before carrying out the titration, care should be taken to cool the final solution if necessary to a temperature not exceeding 25 °C. If the temperature of this solution is

higher because of insufficient cooling of the distillate, the concentration of hydrogen ions will be affected, and hence the end-point of the indicator altered.

## 10 TEST REPORT

The test report shall give all the information necessary for the complete identification of the sample and indicate the result obtained, specifying clearly whether this represents the total nitrogen content of the product as such or the total nitrogen content of the product, relating to the dry material. If the result is expressed as crude protein, the report should also indicate the multiplication factor used to convert the total nitrogen content to crude protein content, i.e. 6,25.

The report shall also indicate whether the method used is that specified in this International Standard and in ISO/R 1871 and indicate any operations not included in ISO/R 1871 and this International Standard or which are regarded as optional, as well as any incidents likely to have influenced the result.