INTERNATIONAL STANDARD 1871

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Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method

Produits agricoles alimentaires — Directives générales pour le dosage de l'azote selon la méthode de Kjeldahl

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FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO Member Bodies). The work of developing International Standards is carried out through ISO Technical Committees. Every Member Body interested in a subject for which a Technical Committee has been set up has the right to be represented on that Committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

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.

Prior to 1972, the results of the work of the Technical Committees were published as ISO Recommendations; these documents are now in the process of being transformed into International Standards. As part of this process, Technical Committee ISO/TC 34 has reviewed ISO Recommendation R 1871 and found it technically suitable for transformation. International Standard ISO 1871 therefore replaces ISO Recommendation R 1871-1971 to which it is technically identical.

ISO Recommendation R 1871 was approved by the Member Bodies of the following countries :

Australia	Greece	Portugal
Austria	Hungary	Romania
Brazil	India	South Africa, Rep. of
Czechoslovakia	Israel	Sweden
Denmark	Netherlands	Turkey
Egypt, Arab Rep. of	New Zealand	United Kingdom
Finland	Peru	U.S.S.R.
France	Poland	

No Member Body expressed disapproval of the Recommendation.

The Member Body of the following country disapproved the transformation of ISO/R 1871 into an International Standard :

United Kingdom

Printed in Switzerland

Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method

0 INTRODUCTION

0.1 The analysis of products of animal or vegetable origin, particularly food products, often includes a determination of the so-called total nitrogen by the Kjeldahl method.

If therefore seems useful, in order to make the results comparable, to recommend a single procedure for this determination.

Experience has shown

a) that different procedures are in use, depending on the products and the operators in the various countries;

b) that these different procedures, when correctly applied, give very similar results.

0.2 As far as can be seen, the spread of the results of the determination of nitrogen, due to the variety of procedures, generally seems less than the spread of the results attributable to the heterogeneity of the products examined. Furthemore, the fact of choosing a special procedure for each type of product to be analysed has the following results :

a) it is undoubtedly satisfactory to those analysts who, since they always analyse the same product, have chosen the procedure to which they are accustomed and which suits them;

b) it compels laboratories which analyse various types of products, and wish to follow International Standards, to multiply their procedures. This obliges them to have available a variety of types of apparatus and reagents and to train personnel in their use, which is contrary to the spirit of standardization.

In addition, for the analysis of compound products, of which each constituent should in principle be analysed by a special procedure, it is necessary to choose a method which will not necessarily be that adopted for each of the constituents of the product. This can only be damaging to the application of International Standards;

c) it means, as often as not, that laboratories which analyse different types of products find themselves unable to use the exact procedures specified because they do not have at their disposal all the necessary types of apparatus and reagents for these various procedures. They are therefore forced either to use their normal procedure (which may not correspond to all the requirements) or to work out a compromise between their own procedure and that specified, and thus to deviate from the International Standards.

0.3 The conclusion reached is that the Kjeldahl method can be standardized in principle, but that it is possible to agree that various forms of apparatus or procedures are equivalent if they give equivalent results.

It has therefore been thought preferable

a) to define the general directions necessary for the correct application of the Kjeldahl method for the determination of nitrogen in agricultural food products; this is the purpose of the present International Standard;

b) to leave it to the International Standards which are specific to certain products to describe detailed procedures which are in accordance with the provisions of the general directions and which can constitute working documents for laboratories.

There are products containing nitrogenous compounds in which the nitrogen cannot be determined by the Kjeldahl method; these are special cases not compatible with the general directions, and other suitable methods should be the subject of special International Standards for these products.

1 SCOPE

This International Standard gives general directions for the apparatus and procedures used for the determination of nitrogen in agricultural food products by the Kjeldahl method. All variants of the procedure should be in accordance with these directions in order to obtain equivalent results.

2 FIELD OF APPLICATION

This International Standard applies to products containing only nitrogenous compounds which are directly determinable by the Kjeldahl method. Products containing, for example, nitrites or nitrates in major amounts require special treatment, although this International Standard can be applied to cured meat products in view of their low residual nitrite and nitrate content.

3 PRINCIPLE

Destruction of organic matter by sulphuric acid in the presence of a catalyst, rendering of the reaction product alkaline, distillation and titration of the liberated ammonia.

4 TEST PORTION

Since many samples of products of animal or vegetable origin (particularly food products) cannot be obtained in a state of perfect homogeneity after preparation in the laboratory, it is advisable to adopt macro-methods.

The test portion, which varies in amount according to the assumed nitrogen content determinable by the Kjeldahl method, shall be representative of the sample and shall contain between 0,005 and 0,2 g of nitrogen and, preferably, more than 0,02 g.

In the case of insufficiently homogeneous products, however, the test portion shall be large (exceeding 1 g) and, if the nitrogen content is high, the determination shall be carried out on an aliquot portion of the liquid resulting from the destruction of organic matter.

The test portion shall be weighed or measured with a precision at least equal to 0,1 %.

Particularly in the case of a viscous liquid or a product in paste form, the test portion can be taken in a small glass container which is placed in the flask, or in a sheet of aluminium, paper or plastics material, which does not yield additional nitrogen or of which the nitrogen content is known.

5 DESTRUCTION OF ORGANIC MATTER

5.1 Sulphuric acid

The acid used shall be practically free from nitrogenous compounds (see 8.2).

If acid of density ρ_{20} = 1,83 to 1,84 g/ml is used, at least 12 ml of acid should be taken for a test portion containing at most 1 g of dry substance and 6 to 12 ml per gram of additional dry substance.

This information is given only as an indication and should be adjusted for each type of product considered. Avoid unnecessary excess.

5.2 Catalysts

A distinction should be drawn between substances intended to raise the boiling point of the liquid during the destruction of organic matter, and catalysts proper which facilitate such destruction. The former substances are generally sodium sulphate or preferably potassium sulphate; they are introduced in sufficient quantity to raise the boiling point to approximately 360 to 380 °C at the end of the digestion.

These substances are frequently mixed in advance with the appropriate catalyst, and then a reagent known as a "compound catalyst" is obtained.

Agreement on the choice of catalyst has proved particularly difficult. Various formulae can be shown in the individual International Standards.

All catalysts which are effective and satisfy the blank tests and the check tests are acceptable.

When the sample is in powder form, it is often advisable to mix the test portion with the catalyst dry, in the Kjeldahl flask, before adding the sulphuric acid.

5.3 Heating

The start of heating is a critical moment in the Kjeldahl method : in many cases foam appears which may rise into the neck of the flask or even escape from it. Particular attention should be paid to this point in the manipulation; moderate heating should be applied at the beginning of the operation. It is sometimes advisable to add an anti-foaming agent (paraffin or various substances which alter the surface tension). It is then essential to ensure that these substances do not yield any additional nitrogen.

If the heat source gives off an intense infra-red radiation, it is often observed that substances (for example, carbohydrates) which generally have a tendency to cause foam, form instead masses of carbonaceous material which then take longer to dissolve but do so without foaming strongly. It may also be advantageous in certain cases to defer the heating, for example overnight.

It is difficult to give a general description of suitable heating sources using gas or electricity. The intensity of heating may be specified to some extent by indicating the time necessary to raise the temperature of a given volume of water, in a flask similar to that used for the test, from 20 °C to boiling point. In practice, heating is adequate if the boiling acid condenses towards the middle of the neck of the usual type of Kjeldahl flask, for example of capacity 300 ml. In all cases, it is essential to avoid overheating of the walls of the flask where they are not in contact with liquid; this can be arranged, for example, by placing the flask on a sheet of asbestos with a hole of diameter slightly less than that of the free surface of the liquid in the flask.

Throughout the heating, it is advisable to place the flask on a support so that its axis is inclined at an angle of 30 to 45° to the vertical.

Many techniques specify agitation of the flask from time to time during destruction of the organic matter. This procedure can often be avoided by placing in the flask a glass ball, for example 5 to 7 mm in diameter.

When the liquid has become clear, the absence of further change of colour does not necessarily indicate complete destruction of the organic matter; the nitrogen in certain resistant compounds, such as lysine, tryptophan or tyrosine, is rendered inorganic only by prolonging the heating for 30 to 90 min after the liquid has become clear. An additional heating of 30 to 40 min is generally sufficient. For any one product, and with a test portion of the same mass, the duration of heating depends both on the source of heat available and on the catalyst selected.

The optimum heating conditions are those which enable the highest results for the determination of nitrogen to be obtained, after having eliminated all sources of error.

During the heating, the mouth of the flask can be partially blocked, for example with a glass bulb with a short stem, or connected to a device for absorbing or aspirating fumes. Such a device should not involve any risk of retaining acid liquid which has been splashed up, or of contamination by residues from an earlier operation.

In all cases, it is advisable during the cooling to take the necessary steps to protect the contents of the flask from any ammonia fumes which may be present in the laboratory.

To summarize, use an electric or gas appliance which does not cause overheating of the walls of the flask not in contact with the liquid, and which is capable of ensuring sufficient boiling for the acid to condense towards the middle of the neck of the usual type of Kjeldahl flask; continue heating for at least 30 min after the liquid has become clear and no longer changes colour.

5.4 Precipitation of mercury

If the catalyst used contains mercury, the mercury shall be precipitated before the distillation of the ammonia is carried out.

Sodium hypophosphite or potassium hypophosphite is the best reagent for precipitating mercury. This reagent shall be introduced in the dry state, after diluting the medium and before making it alkaline.

In practice, 1 g of sodium hypophosphite or of potassium hypophosphite suffices for the precipitation of up to 1 g of mercury.

NOTE – The precipitation of mercury by alkaline sulphides or thiosulphates involves a risk of the release of hydrogen sulphide or sulphur dioxide into the atmosphere in the apparatus if, during mixing, these reagents are temporarily in contact with an acid area of the medium. These acid gases, if passing directly into the distillate, then neutralize part of the ammonia, and this causes an error leading to a low result.

6 DISTILLATION OF AMMONIA

6.1 Apparatus

6.1.1 The various procedures known at the present time describe a great variety of distillation apparatus. A distinction can be made between

a) apparatus which allows distillation to be carried out without transfer of the sulphuric acid solution contained in the digestion flask;

b) apparatus which requires transfer of the acid solution, either as a whole or by the removal of an aliquot portion.

6.1.2 The distillation of ammonia can be carried out by various methods :

a) by simple distillation after dilution with water;

b) by steam distillation, possibly with thermal insulation or auxiliary heating of the vessel containing the solution to be distilled;

c) by distillation with superheated steam, which does not require auxiliary heating.

6.1.3 Every apparatus shall include a device for condensing steam and collecting the ammonia vapour.

All these various types of apparatus are admissible, if they satisfy the check tests described in 8.3.

The apparatus shall have the following characteristics :

a) it shall prevent any loss of ammonia, either by volatilization in the atmosphere at the moment of adding alkali, or by leakage during distillation;

b) it shall ensure complete distillation of the ammonia;

c) it shall prevent any accidental carry-over of the sodium hydroxide solution, droplets of which shall be retained by means of an efficient trap.

6.2 Addition of alkali

Whatever type of apparatus is used, it is necessary to dilute the acid liquor with water and then to make it alkaline by the addition of a sufficient quantity of sodium hydroxide solution.

If the sulphuric acid used has a density $\rho_{20} = 1{,}83$ to 1,84 g/ml, and if the alkali added is a sodium hydroxide solution (free from carbonate) of density $\rho_{20} = 1{,}33$ g/ml (about 30 % (*m*/*m*)), it is necessary to add at least 3,5 ml of sodium hydroxide solution per millilitre of sulphuric acid remaining in the flask after destruction of the organic matter, or per millilitre of the aliquot portion taken for distillation, or 2 ml of sodium hydroxide solution per gram of acid. If the amount of sulphuric acid remaining in the flask is difficult to estimate, an amount of sodium hydroxide solution corresponding to the total amount of sulphuric acid shall be taken.

For example, for 12 ml of sulphuric acid, add 45 to 50 ml of sodium hydroxide solution.

This addition should be made with care, since the mixture becomes strongly heated, and with certain types of apparatus there is a risk of loss of ammonia or even of the breakage of the apparatus owing to splashing of hot caustic liquid.

6.3 Distillation

Carry out the distillation according to the conditions of use of the apparatus in question.

Ensure that the distillation of the ammonia is complete, and does not include any excess due to the carry-over of alkaline liquid. This is the reason for recommending simultaneous titration. (See 7.1.)

However, various procedures recommend the use of different criteria for the end of distillation : for example, the volume of distillate collected, the volume of undistilled residue or the duration of the operation. These criteria have no direct relation to the essential operation, i.e. the quantitative distillation of ammonia. They can be adopted only if exploratory tests have shown that this distillation is complete under the conditions indicated. It is always preferable to verify the end of distillation for each test.

7 TITRATION

7.1 Titration procedure

7.1.1 *Simultaneous titration*

The simultaneous titration of the ammonia during distillation is recommended since it facilitates verification of the end of distillation.

It may be carried out by titrating the ammonia in the distillate, as it is carried over, either in distilled water, or, preferably, by collecting the distillate in a boric acid solution.

In the latter case it is sufficient to use 10 to 25 ml of an aqueous solution of boric acid of strength approximately 4 %.

Simultaneous titration also makes it possible to observe whether any carry-over of the sodium hydroxide solution has taken place, in which case the titration ceases to be significant. If this happens the result is vitiated and the analysis should be repeated.

A blank test carried out by distilling a mixture of water and sodium hydroxide can confirm the existence of this carry-over.

Overheating of this liquid by an insufficiently cooled distillate shall be avoided; the temperature shall not rise above 25 °C.

7.1.2 *Titration after distillation*

Titration after distillation may be carried out either directly in the boric acid solution, or by back titration.

Back titration is carried out by collecting the ammonia in a standard volumetric solution of a strong acid, the excess of which is determined later. It is also necessary to ensure that distillation is complete under the test conditions.

The errors mentioned above (incomplete distillation of the ammonia and carry-over of sodium hydroxide solution), which act in opposite directions, may compensate one another to a considerable extent and thus remain undetected.

The strength of the acid solution used shall be not less than 0,02 N and not more than 0,2 N. This strength shall be selected in such a way as to use, for preference, a volume of standard solution between 5 and 50 ml.

7.2 Titration indicator

Various colour indicators have been proposed. Methyl red is one of the most widely used, and bromocresol green can be equally recommended. The indicator known as "Tashiro", i.e. a methyl red solution to which methylene blue has been added, is also suitable, provided that the proportion of blue colour is adjusted so as to obtain a neutral grey colour at pH 5,5.

Any indicator, or any electrometric device giving satisfactory results in the check tests, is admissible.

8 TESTS

8.1 General

The balances, the weights and the volumetric glassware are assumed to be accurate. The concentrations of the solutions used are assumed to be known exactly.

First carry out a blank test : verification of the reagents.

Then carry out check tests : verification of the apparatus and of the procedure.

One blank test and at least one check test shall be included in each series of determinations.

The blank test shall be carried out each time a reagent is renewed.

8.2 Blank test

The blank test includes all the stages of the procedure, and is carried out by replacing the test portion by an equivalent amount of an organic substance free from nitrogen (for example sucrose) but such as to promote the reduction of any nitric or nitrous compounds that may be present in the reagents. It should give a result which is practically zero (error of the order of one drop of the standard solution selected).

Otherwise, it is necessary to make the corresponding correction if the error is slight or, preferably, to divide the test to determine which reagent is adding nitrogen or whether the apparatus is causing carry-over of sodium hydroxide solution. The defect detected should then be remedied.

8.3 Check tests

8.3.1 *Test of the distillation apparatus*

Place in the apparatus a known quantity of an ammonium salt, for example 10 ml of a 0,1 N solution of ammonium sulphate. Make alkaline, distil, titrate.

A low result can be attributed to incomplete distillation or to leakage in the apparatus.