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R 1871

AGRICULTURAL FOOD PRODUCTS

GENERAL DIRECTIONS FOR THE DETERMINATION OF NITROGEN BY THE KJELDAHL METHOD

1st EDITION

May 1971

For each individual country the only valid standard is the national standard of that country.

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BRIEF HISTORY

The ISO Recommendation R 1871, *Agricultural food products – General directions for the determination of nitrogen by the Kjeldahl method,* was drawn up by Technical Committee ISO/TC 34, *Agricultural food products,* the Secretariat of which is held by the Magyar Szabványügyi Hivatal (MSZH).

Work on this question led to the adoption of Draft ISO Recommendation No. 1871, which was circulated to all the ISO Member Bodies for enquiry in January 1970. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

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

No Member Body opposed the approval of the Draft.

This Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided to accept it as an ISO RECOMMENDATION.

ISO Recommendation R 1871 May 1971

AGRICULTURAL FOOD PRODUCTS

GENERAL DIRECTIONS FOR THE DETERMINATION OF NITROGEN BY THE KJELDAHL METHOD

INTRODUCTION

I. 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.

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

Experience has shown

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

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

II. 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. Furthermore, the fact of choosing a special procedure for each type of product to be analysed has the following results :

(1) 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;

(2) it compels laboratories which analyse various types of products, and wish to follow ISO Recommendations, 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 ISO Recommendations;

(3) 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 ISO Recommendations.

III. 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

(1) 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 ISO Recommendation;

(2) to leave it to the ISO Recommendations 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 ISO Recommendations for these products.

1. SCOPE

This ISO Recommendation 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 ISO Recommendation 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 ISO Recommendation 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, should be representative of the sample and should 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 should be large (exceeding 1 g) and, if the nitrogen content is high, the determination should be carried out on an aliquot portion of the liquid resulting from the destruction of organic matter.

The test portion should be weighed or measured with a precision at least equal to 1 in 1000.

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 should be practically free from nitrogenous compounds (see clause 8.2, Blank test).

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 gramme 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 ISO Recommendations.

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 minutes after the liquid has become clear. An additional heating of 30 to 40 minutes 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 minutes after the liquid has become clear and no longer changes colour.

5.4 Precipitation of mercury

If the catalyst used contains mercury, the mercury should 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 should 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

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

(2) 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 :

(1) by simple distillation after dilution with water;

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

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

6.1.3 Every apparatus should 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 clause 8.3.

The apparatus should have the following characteristics :

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

(2) it should ensure complete distillation of the ammonia;

(3) it should prevent any accidental carry-over of the sodium hydroxide solution, droplets of which should 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 ρ_{20} = 1.83 to 1.84 g/ml, and if the alkali added is a sodium hydroxide solution (free from carbonate) of density ρ_{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 gramme 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 should 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.