INTERNATIONAL STANDARD ISO 5378

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Starches and derived products — Determination of nitrogen content by the Kjeldahl method — Spectrophotometric method

Amidons, fécules et produits dérivés — Dosage de l'azote selon la méthode de Kjeldahl — Méthode spectrophotométrique

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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 5378 was developed by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products)*, and was circulated to the member bodies in March 1976.

It has been approved by the member bodies of the following countries :

Australia	Germany	Romania
Austria	Hungary	Spain
Chile	Ireland	Turkey
Czechoslovakia	Mexico	United Kingdom
Finland	Netherlands	Yugoslavia
France	Philippines	

The member body of the following country expressed disapproval of the document on technical grounds :

Poland

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Starches and derived products — Determination of nitrogen content by the Kjeldahl method — Spectrophotometric method

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a spectrophotometric method for the determination, by the Kjeldahl method, of the nitrogen content of starch and its derived products whose presumed nitrogen content is less than 0,025 % (*m/m*).[1)]

NOTE — In starches and their derived products to which nitrogenous materials have not been added, the nitrogen is present essentially in the form of protein and/or amino acids.

2 REFERENCES

ISO 1227/Add. 2, *Starch, including derivatives and by-products — Vocabulary, Addend.*

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

ISO 3188, *Starches and derived products — Determination of nitrogen content by the Kjeldahl method — Titrimetric method.*

3 DEFINITION

nitrogen content : The value found using the procedure specified. It includes the nitrogen content of free amino acids, of compounds producing amino acids on hydrolysis and of ammonium compounds. It does not include the nitrogen of nitrate and nitrite radicals, the nitrogen attached directly to another nitrogen atom or the nitrogen attached to an oxygen atom.

4 PRINCIPLE

Destruction of organic matter by sulphuric acid in the presence of a compound catalyst[2)], alkalization of the reaction products, distillation of the liberated ammonia and collection in a sulphuric acid solution, followed by spectrophotometry, of the ammonium salt formed after the addition of the Nessler reagent.

5 REAGENTS

The reagents shall be of recognized analytical quality. Ammonia-free distilled water or water of at least equivalent purity shall be used.

5.1 Sulphuric acid, concentrated, ρ_{20} 1,84 g/ml [96 % (*m/m*)].

5.2 Sodium hydroxide, solution 30 % (*m/m*), ρ_{20} 1,33 g/ml.

NOTE — This solution may be more concentrated.

5.3 Compound catalyst[3)], consisting of, for example

- potassium sulphate : 97 g;
- copper(II) sulphate, anhydrous : 3 g.

5.4 Ammonium sulphate, ammonium oxalate or **ammonium chloride.**

5.5 Sulphuric acid, approximately 0,1 N standard volumetric solution.

5.6 Nessler reagent, prepared as follows at least 2 days before use :

Dissolve 100 g of mercury(II) iodide and 70 g of potassium iodide in 100 ml of water. Dissolve 224 g of potassium hydroxide in 700 ml of water in a 1 000 ml one-mark volumetric flask and allow to cool to ambient temperature. Add the mercury(II) iodide/potassium iodide solution slowly, with stirring, to the potassium hydroxide solution. Dilute to the mark with water and mix. Allow to stand for at least 2 days before using.

NOTE — The reagent should be kept in a brown glass bottle. If it is kept in an air-tight bottle and in the shade, it can be used even after 1 year if care is taken to leave the sediments undisturbed when it is re-used.

1) For products whose presumed nitrogen content is greater than 0,01 % (*m/m*), see ISO 3188.

2) See ISO 1871.

3) See ISO 1871, sub-clause 5.2.

6 APPARATUS

Usual laboratory equipment and, in particular :

6.1 Kjeldahl flask, of suitable capacity, usually 500 to 800 ml, preferably with a ground glass joint, and provided with a pear-shaped glass bulb fitting loosely in the top of the neck of the flask.

6.2 Digestion stand, on which the Kjeldahl flask (6.1) can be heated in an inclined position in such a way that heat is applied only to that part of the flask wall which is below the liquid level at all stages.

6.3 Distillation or steam distillation apparatus, with a 200 ml graduated dropping funnel and an efficient splash head, the latter connecting the Kjeldahl flask (6.1) to the condenser.

Any apparatus that satisfies the control tests given in ISO 1871 is permitted.

6.4 Conical flasks, capacity 100 ml.

6.5 One-mark volumetric flask, capacity 200 ml, with a plain neck, complying with the requirements of ISO 1042, class A.

6.6 Pipettes, of suitable capacity, complying with the requirements of ISO 648, class A.

6.7 Spectrophotometer, capable of being adjusted at a wavelength of 430 nm and provided with appropriate cells whose optical path length shall be stated in the test report.

6.8 Mechanical grinder or **mortar.**

6.9 Sieve, with a nominal mesh opening of 0,6 mm, complying with the requirements of ISO 565.

6.10 Analytical balance.

7 PROCEDURE

7.1 Preparation of the test sample

Mix the sample thoroughly and rapidly by shaking or stirring with a spatula in the sample container[1]. If the sample container is too small for this purpose, transfer the entire sample to another predried container of a suitable size to facilitate mixing.

It may be necessary to grind the sample, in which case it must pass through the sieve (6.9) without leaving any residue.

7.2 Test portion

Weigh, to the nearest 0,001 g, 2 to 5 g (mass m_0) of the test sample (7.1), according to the presumed nitrogen content, and transfer to the predried Kjeldahl flask (6.1), taking care that none of the product adheres to the inner wall of the neck of the flask.

In the case of a viscous liquid or a product in paste form, the test portion may be weighed in a small glass container or on a sheet of aluminium, paper or plastics which does not yield nitrogen, or whose nitrogen content is known, and which is left in the flask. In the case of a container which yields nitrogen, this should be taken into account in the blank test (7.8).

7.3 Destruction of organic matter

Add 3 g of the compound catalyst (5.3) and, using a suitable measuring cylinder, add the appropriate volume, in millilitres, of the concentrated sulphuric acid (5.1), calculated by the formula $20 + 4\,m_0$, in such a way that the acid rinses the inner wall of the neck of the flask.

Mix the contents of the flask by swirling the flask gently until the mixture is free from lumps and the test portion is completely wetted. In order to avoid super-heating, add a boiling aid (for example glass beads). Insert the pear-shaped glass bulb (see 6.1) in the neck of the flask and place it in an inclined position on the digestion stand (6.2).

Heat with care until the liquid in the flask boils gently. Continue to heat for 1 h after the liquid becomes clear. In the case of digestion apparatus heated by gas, ensure that the flame does not extend beyond the part of the flask filled with liquid, in order to avoid loss of nitrogen.

7.4 Distillation

Allow the contents of the flask to cool and rinse the pear-shaped glass bulb and the inner neck of the flask with a few millilitres of water, allowing the rinsings to run into the flask. Add, with care, between 50 and 150 ml of water (according to the apparatus used), whilst swirling the contents of the flask. Connect the flask to the distillation or steam distillation apparatus (6.3), previously freed from ammonia by steaming.

Adjust the lower end of the condenser so that it just touches the bottom of the one-mark volumetric flask (6.5), containing 25 ml of the sulphuric acid solution (5.5). Render the digestion liquid alkaline by slowly adding, through a graduated separating funnel (see 6.3) placed in the neck of the flask, 120 ml of the sodium hydroxide solution (5.2), ensuring that the neck of the funnel does not become empty. Mix well, then turn on the condenser water and start heating; the ammonia then begins to be carried over.

1) In the case of glucose syrup, remove the surface layer (about 5 mm) before mixing.

During distillation ensure that steam generation is kept constant. Distillation is complete when about 150 ml of liquid have been collected in 20 to 30 min.

Turn off the heat and lower the conical flask. Allow the condenser to drip for a few minutes into the flask and rinse the tip of the condenser with water, collecting the rinsings in the conical flask. Dilute to the mark with water and mix.

7.5 Preparation of the calibration curve

Prepare a suitable series of standard matching solutions containing at least five different and known concentrations of an ammonium salt (5.4). Using the pipette (6.6), transfer 50 ml of each of these standard solutions into a series of separate 100 ml conical flasks (6.4) and 50 ml of water into another one-mark 100 ml conical flask (6.4). The last mentioned corresponds to a zero concentration of the ammonium salt for the calibration curve.

Add by pipette 1,0 ml of the Nessler reagent (5.6) to each flask and mix separately.

Allow to stand for 10 min and measure the absorbance of each of these solutions, using the spectrophotometer (6.7) adjusted at a wavelength of 430 nm.

Plot the calibration curve showing the absorbance as a function of the number of micrograms of nitrogen.

NOTES

1 Between 0 and 240 µg of nitrogen for 50 ml (about 0 to 5 000 µg of nitrogen for 1 000 ml) of solution the calibration curve is a straight line.

2 Each point on the calibration graph should be the arithmetic mean of two determinations.

7.6 Spectrophotometry

Using a suitable pipette (6.6), transfer a volume (V) of the distillate obtained in 7.4 and containing no more than 175 µg of nitrogen (in general between 25 ml and 50 ml, according to the expected nitrogen content of the product) into a 100 ml conical flask (6.4). If necessary, make the volume up to 50 ml with water. Add by pipette 1,0 ml of the Nessler reagent (5.6) and mix. Allow to stand for 10 min and measure the absorbance of the solution, using the spectrophotometer (6.7) adjusted at a wavelength of 430 nm.

7.7 Number of determinations

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

7.8 Blank test

Carry out a blank test on the reagents only, following the procedure specified in 7.4 and 7.6 using 50 ml of distillate. If the test portion has been weighed in a container which yields nitrogen (see 7.2), carry out the blank test using an identical container and include the procedure specified in 7.3.

7.9 Check tests

Carry out the check tests specified in ISO 1871.

8 EXPRESSION OF RESULTS

The nitrogen content in the sample is given, as a percentage by mass, by the formula.

$$\left(m_1 \times \frac{200}{V} - m_2 \times \frac{200}{50}\right) \times \frac{1}{10^6} \times \frac{100}{m_0}$$

$$= \left(\frac{m_1}{V} - \frac{m_2}{50}\right) \times \frac{1}{50\, m_0}$$

where

m_0 is the mass, in grams, of the test portion (7.2);

m_1 is the mass, in micrograms, of nitrogen in the aliquot portion (V ml) of the distillate from the digestion of the test portion (7.2), determined from the calibration curve;

m_2 is the mass, in micrograms, of nitrogen in the 50 ml aliquot portion of the distillate in the blank test (7.8), determined from the calibration curve;

V is the volume, in millilitres, of the aliquot portion of the distillate taken in 7.6.

Express the result as the arithmetic mean of the two determinations.

9 TEST REPORT

The test report shall indicate the method used, the optical path length of the cell and the results obtained. It shall also mention all operating conditions that are not specified in this International Standard, or are regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details required for complete identification of the sample.

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