INTERNATIONAL STANDARD 3188

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

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

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

First edition — 1978-08-15

UDC 664.2 : 546.17 : 543.24 **Ref. No. ISO 3188-1978 (E)**

Descriptors : starches, chemical analysis, determination of content, nitrogen, volumetric analysis, Kjeldahl method.

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.

International Standard ISO 3188 was developed by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products)*, and was circulated to the member bodies in August 1975.

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

Australia	Iran	Thailand
Czechoslovakia	Netherlands	Turkey
France	Poland	United Kingdom
Germany	Romania	Yugoslavia
Hungary	South Africa, Rep. of	

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

U.S.A.

Printed in Switzerland

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

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a titrimetric method for the determination, by the Kjeldahl method, of the nitrogen content of starch and its derived products whose presumed nitrogen content is greater than 0,01 % (*m/m*).[1)]

NOTE — In starches and 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.*

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

ISO 5378, *Starches and derived products — Determination of nitrogen content by the Kjeldahl method — Spectrophotometric 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 boric acid solution, followed by titration with a standard volumetric sulphuric acid solution.

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 Boric acid, 20 g/ solution.[3)]

5.4 Compound catalyst[4)], consisting of, for example :

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

5.5 Sulphuric acid, approximately 0,02 N or 0,1 N standard volumetric solution.

5.6 Colorimetric indicator, prepared by mixing 2 parts by volume of a cold saturated solution of neutral methyl red in 50 % (*V/V*) ethanol with 1 part by volume of a 0,25 g/l solution of methylene blue in 50 % (*V/V*) ethanol.

Store the indicator in a brown glass bottle.

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.

1) For products whose presumed nitrogen content is less than 0,025 % (*m/m*), see ISO 5378.

2) See ISO 1871.

3) For anticipated low nitrogen contents, a less concentrated solution may be needed in order to obtain greater accuracy.

4) See ISO 1871, sub-clause 5.2.

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 Burette, 25 ml with 0,05 ml graduations, or 10 ml with 0,01 ml graduations.

6.5 Mechanical grinder or **mortar.**

6.6 Sieve, with nominal mesh openings of 0,6 mm, complying with the requirements of ISO 565.

6.7 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 shall all pass through the sieve (6.6) without leaving any residue.

7.2 Test portion

Weigh, to the nearest 0,001 g, up to 10 g (mass m) 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 (7.6).

7.3 Destruction of organic matter

Add 10 g of the compound catalyst (5.4) and, using a suitable measuring cylinder, add the appropriate volume, in millilitres, of the concentrated sulphuric acid (5.1), calculated by the formula $20 + 4\,m$, 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 and titration

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 200 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 a 500 ml conical flask containing a known volume (varying between 25 and 50 ml) of the boric acid solution (5.3) and 3 to 5 drops of the titration indicator (5.6). Render the digestion liquid alkaline by slowly adding, through the graduated separating funnel (see 6.3) placed in the neck of the flask, between 150 and 200 ml of the sodium hydroxide solution (5.2), ensuring that the stem 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.

The indicator contained in the flask turns immediately to its alkaline colour.

During distillation, ensure that steam generation is kept constant. Distillation is complete when 200 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.

The liquid contained in the flask should be green.

Titrate the contents of the flask with either 0,02 N or 0,1 N sulphuric acid solution (5.5), using a 10 ml or 25 ml burette (6.4) as appropriate, until the colour of the contents turns reddish violet.

7.5 Number of determinations

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

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

7.6 Blank test

Carry out a blank test on the reagents only. 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.

7.7 Check tests

Carry out the check tests specified in ISO 1871.

8 EXPRESSION OF RESULTS

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

$$0{,}014\,01 \times T \times (V_1 - V_0) \times \frac{100}{m}$$

$$= \frac{1{,}401\,T\,(V_1 - V_0)}{m}$$

where

T is the normality of the sulphuric acid solution (5.5) used in the two titrations;

V_0 is the volume, in millilitres, of the sulphuric acid solution used in the blank test (7.6);

V_1 is the volume, in millilitres, of the sulphuric acid solution used in the determination (7.4);

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

Express the result as the arithmetic mean of the two determinations if the volumes of the sulphuric acid solution used do not differ by more than 0,1 ml. Otherwise, repeat the determinations on the same test sample.

9 TEST REPORT

The test report shall indicate the method used 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.

This page intentionally left blank