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Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method

Produits alimentaires et aliments des animaux — Lignes directrices générales pour le dosage de l'azote selon la méthode de Kjeldahl

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 1871 was prepared by Technical Committee ISO/TC 34, *Food products*.

This second edition cancels and replaces the first edition (ISO 1871:1975), which has been technically revised.

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Introduction

The analysis of products of animal or plant origin, such as those used in food and feed products, often includes determining their nitrogen content according to the Kjeldahl method.

This method can be standardized in principle, as it is generally accepted that different apparatus or operating methods are equivalent if their results are similar.

The purpose of this document is to describe the various stages of the method, the associated critical points and the minimum objectives to be achieved to ensure that the method is applied correctly.

This document provides general guidelines; it is not intended to replace existing International Standards which are in use.

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Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard provides general guidelines for the determination of nitrogen by the Kjeldahl method. It applies to food and feed products containing nitrogenous compounds that can be directly determined by the Kjeldahl method.

NOTE This measurement principle does not take into account the nitrogen from nitrates and nitrites.

2 Principle

Digestion of a test portion with concentrated sulfuric acid in the presence of catalysts to convert the organic nitrogen into ammonium sulfate. Excess sodium hydroxide is added to the cooled digest to release the ammonia. The released ammonia is distilled into an excess of boric acid solution and then titrated with a standard solution of sulfuric or hydrochloric acid. The nitrogen content is calculated from the quantity of ammonia produced.

NOTE In the following text, the term “nitrogen” refers to organic nitrogen.

3 Reagents

Use only reagents of recognized analytical grade, *unless otherwise specified*, and distilled or demineralized water or water of equivalent purity.

3.1 Sulfuric acid, virtually free from nitrogenous compounds and of mass density ρ_{20} = 1,83 g/ml to 1,84 g/ml.

3.2 Catalysts (see 5.2.1).

3.3 Boric acid solution (10 g/l to 40 g/l depending on the apparatus used). If using the colorimetric end-point titration, boric acid solution shall contain indicator (the pH or colour of this mixed solution shall be adjusted before use).

3.4 Standard hydrochloric acid (0,02 mol/l to 0,50 mol/l) or **sulfuric acid solution** (0,01 mol/l to 0,25 mol/l). The titre of the solution, c_t, shall be known to at least within 0,001 mol/l.

3.5 Indicators, which should change colour between pH 4 and pH 5.

NOTE Various indicators are available. A methyl red and bromocresol green mixed indicator is most commonly used. Ready-to-use boric acid solutions containing mixed indicators are available.

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3.6 Hydrogen peroxide (H_2O_2), min. 30 % mass fraction.

3.7 Sodium hydroxide solution, min. 30 % mass fraction.

3.8 Antifoaming agents.

EXAMPLE Silicone, liquid paraffin.

3.9 Ammonium sulfate or **ammonium chloride** (minimum purity 99,9 %).

Immediately before use, dry the ammonium sulfate or ammonium chloride at 104 °C ± 4 °C for at least 2 h. Allow it to cool at ambient temperature in a desiccator.

NOTE Solutions of known concentration can be used.

3.10 Tryptophan or **acetanilide** or **lysine hydrochloride** (minimum purity 99 % mass fraction).

These reagents should be kept away from humidity.

WARNING — Do not dry these reagents in an oven before use.

3.11 Sucrose, with nitrogen content less than a mass fraction of 0,002 %.

WARNING — Do not dry sucrose in an oven before use.

4 Apparatus and materials

Usual laboratory apparatus and, in particular, the following.

4.1 Analytical balance, capable of weighing to the nearest 0,001 g.

4.2 Digestion, steam distillation and titration systems.

They are used to perform the operations described in Clause 5 and to ensure that the performance objectives described in 5.5.3 and 5.5.4 are met.

4.3 Boiling point regulators (if needed), for example pumice grains, glass beads, aluminium oxide (corundum) or silicon carbide.

4.4 Weighing paper or **medium**, free from nitrogenous compounds and suitable for the test portion and type of product.

5 Operating method

NOTE According to the nature of the sample, it may be necessary to prepare the test portion in advance to obtain a homogeneous sample (grinding, homogenization, etc.).

5.1 Test portion

The test portion, the quantity of which depends on the presumed nitrogen content determined by the Kjeldahl method, shall be representative of the sample and contain between 0,005 g and 0,2 g of nitrogen.

The test portion can be obtained by weighing with the analytical balance (4.1), to give mass, m, in grams or by using a pipette, to give volume, V_t, in millilitres.

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The test portion can be inserted into the tube directly or via a support (4.4).

The quantity of test portion can be adjusted according to the composition of the product under test and the quantity of sulfuric acid (see 5.2.2).

5.2 Digestion

5.2.1 Catalysts

It is important to differentiate between the substances used to raise the boiling point of the liquid during digestion and the catalysts themselves that facilitate digestion. The former are usually potassium sulfate or, possibly, sodium sulfate. They are introduced in sufficient quantity to raise the boiling point of the acid to between 380 °C and 430 °C. The most commonly used catalyst is copper in the form of copper sulfate alone or mixed with titanium oxide.

The optional addition of hydrogen peroxide (3.6) on the basis of 3 ml to 5 ml per tube prior to heating accelerates digestion, but should be used with the utmost care to ensure that no nitrogen is lost in the form of vapour. Moreover, great care should be taken when adding hydrogen peroxide to the tubes, as this causes a strong exothermic reaction.

The quantity of potassium sulfate provided by the catalyst should not be less than 7 g.

Depending on the sectors of activity, various compositions are used. They should meet the requirements of the blank test (5.5.2) and the control tests (5.5.3 and 5.5.4).

Operators should handle selenium-based catalysts and waste conditions with care.

NOTE Ready-to-use composite catalysts are available on the market (for example in tablet or pellet form).

5.2.2 Addition of acid

It is important to use a sufficient quantity of sulfuric acid to ensure digestion after:

— acid consumption by the organic matter of the sample, bearing in mind the fact that 1 g of fat consumes 10 ml of sulfuric acid, 1 g of protein consumes 5 ml of sulfuric acid, 1 g of carbohydrate consumes 4 ml of sulfuric acid;

— acid consumption by the reagents (salts);

— acid losses by evaporation.

The addition of 20 ml to 25 ml of acid (3.1) is generally sufficient for good digestion and to maintain excess acid at the end of the reaction.

5.2.3 Heating

WARNING — The following operations should be performed under a very well ventilated fume hood.

The manufacturer's instructions relating to the use of the equipment should generally be followed. The digestion system should be made homogeneous, for example by creating a thermal or digestion efficiency process diagram (5.5.3).

Foam-producing agents should be brought to boiling point by increasing the temperature gradually or in steps. Three to four drops of antifoaming agent per tube (3.8) can also be used.

For “dry” products (i.e. with no visible wetness), the tubes can be placed directly in a preheated unit.

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Acid fumes shall be removed with an extraction system suitable for the equipment used. Excessive extraction may cause crystallization and a loss of nitrogen (see Annex A).

In all cases, the digestion temperature and time should be determined to meet the requirements of the digestion control test (5.5.3).

NOTE 1 Heating at 420 °C for two hours is appropriate for numerous matrices.

The digest obtained should be clear and free from black particles.

At the end of the digestion process, allow the tubes to cool away from any possible contamination. At this stage, the test portions can be stored and distilled later.

NOTE 2 Dilution step with water as described in 5.3.1 may be done at this stage in order to avoid crystallization.

5.3 Ammonia distillation

5.3.1 Alkalinization

Dilute the digest with water, and then alkalise by adding at least 3,5 ml of sodium hydroxide solution (3.7) per millilitre of sulfuric acid (3.1) used for the digestion process.

NOTE The volume of the added sodium hydroxide solution (3.7) may be lower if its mass fraction is higher than 30 %.

WARNING — Care should be taken when adding the solution, as the medium becomes very hot.

5.3.2 Distillation

Perform the distillation with the apparatus under consideration in its usual condition. Collect the distillate in the boric acid solution (3.3), which shall contain the indicator (3.5). Adjust the pH until there is a change of colour to grey (bromocresol green + methyl red indicator) before beginning the distillation.

There are several criteria for determining when the distillation process is finished, for example, when a specific volume of distillate has been collected, after a fixed distillation time, and so on. Ensure that in compliance with the control tests (5.5), the ammonia distillation is complete and that there is no excess by entrainment of the alkaline liquid.

5.4 Titration

The distillate obtained is titrated with sulfuric (3.1) or hydrochloric acid (3.4); this can be done simultaneously or after distillation. Post-distillation titration should be performed as soon as possible after distillation.

There are two methods of detecting the end point.

— *By visual colorimetry or using an optical measurement system*: The end point is reached when the indicator changes colour. In the case of visual colorimetry, it is important to titrate each test referring to the conditions obtained in the blank test.

— *By potentiometric analysis with a pH measurement system*: Depending on the equipment or operating methods, the end point may be a fixed pH (generally pH 4,6, which corresponds to the inflection point of the titration curve), the pH obtained in the blank test, or the original pH of the boric acid solution.

In both cases, the validity of the titration operations should be checked as described in 5.5.4.

Record the volumes of titrant obtained: V_0 for the blank test and V_1 for the samples.

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5.5 Control tests

5.5.1 General

One blank test and at least one distillation control test and one digestion control test should be included in each set of nitrogen determination tests.

5.5.2 Blank test

Perform a blank test using the operating method described above, replacing the liquid test portion with the same volume of water and adding the appropriate quantity of sucrose (3.11).

NOTE In blank and digestion control tests, the sucrose is used as organic matter to consume a quantity of sulfuric acid equivalent to that of a test portion during digestion.

5.5.3 Digestion test

Perform a digestion control test using the operating method described above, replacing the test portion with the same quantity of tryptophan or acetanilide or lysine hydrochloride (3.10) as the quantity of nitrogen in the samples and adding the appropriate quantity of sucrose (3.11).

Calculate the percentage mass fraction of nitrogen recovered, which should be between 98 % and 101 %.

5.5.4 Distillation-titration tests

Perform a blank distillation-titration test using the operating method described in 5.3, but without a test portion. The volume obtained should be subtracted from that of the distillation-titration test.

Perform a distillation-titration control test under the same conditions on a test portion of ammonium salt (3.9) corresponding to the quantity of nitrogen in the samples.

Calculate the percentage mass fraction of nitrogen recovered, which should be between 99 % and 101 %.

6 Expression of results

The nitrogen content, expressed as a percentage mass fraction or in grams per 100 ml, is equal to:

$$\frac{(V_1 - V_0) \times c_t \times 14 \times 100}{m \times 1000}$$

or

$$\frac{(V_1 - V_0) \times c_t \times 14 \times 100}{V_t \times 1000}$$

where

m is the mass, in grams, of the test portion;

c_t is the titre, in moles per litre, of the hydrochloric acid or sulfuric acid;

V_0 is the volume, in millilitres, of hydrochloric acid or sulfuric acid used in the blank test titration;

V_1 is the volume, in millilitres, of hydrochloric acid or sulfuric acid used in the test portion titration;

V_t is the volume, in millilitres, of the test portion.

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Annex A
(informative)

Potential sources of error

Faults noted	Causes	Solutions proposed
1. During digestion		
Too much spray or foam	Too rapid a rise in temperature	Reduce the heating rate or adjust the steps
	Fat or sweet sample tests	Use an antifoaming agent
	Too high a volume of liquid samples	Use a smaller test portion
Black particles in the digest	Inappropriate digestion time/temperature	Optimize the conditions: check the digestion (5.5.3)
		Check the sample/acid/catalyst proportions
Pellet crystallization	Loss of acid due to a too powerful fume extraction system	Reduce the extraction rate: it can be reduced as soon as the white fumes disappear
		Check the sample/acid/catalyst proportions
2. During distillation and nitrogen content determination		
Distillation-titration test result too low	Loss of ammonia	Check the apparatus for tightness (seals and glass instruments)
	Insufficient boric acid	Increase the concentration or volume of the boric acid solution
	Ammonia entrainment incomplete	Increase the distillation time
	Incorrect acid titre measurement	Titrate the acid
	Blank distillation-titration test result too high	Perform a new blank test
Distillation-titration test result too high	Incorrect acid titre measurement	Titrate the acid
	Pollution due to ammonia vapour	Avoid handling ammonia in the vicinity
	Entrainment of sodium hydroxide in the distillate	Reduce the volume of water added before distillation
Digestion test result too low	Inappropriate digestion time/temperature	Optimize the conditions: check the digestion (5.5.3)
		Check the sample/acid/catalyst proportions

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