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Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle

Lait et produits laitiers — Détermination de la teneur en azote — Méthode pratique par combustion selon le principe de Dumas

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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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14891 | IDF 185 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

Annexes A, B, C and D are for information only.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of National Committees casting a vote.

International Standard ISO 14891/IDF 185 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Nitrogen compounds*, of the Standing Committee, *Main compounds of milk*, under the aegis of its project leader, Prof. H. Frister (DE).

Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle

1 Scope

This International Standard specifies a routine method for the determination of the total nitrogen content of milk and milk products.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 8968-1 | IDF 20-1, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

3.1
nitrogen content
mass fraction of the total nitrogen determined by the procedure specified in this International Standard

NOTE The nitrogen content is expressed as a percentage by mass.

4 Principle

A test portion is heated to destruction in a combustion tube at high temperature (900 °C to 1 200 °C) in an oxygen atmosphere according to the Dumas principle. All interfering components are removed from the resulting gas mixture. The nitrogen compounds in the test portion are converted to molecular nitrogen, followed by quantitative determination using a thermal conductivity detector. The nitrogen content is calculated using a microprocessor.

5 Reagents

Use only reagents of recognized analytical grade, or reagents of equivalent purity as specified by instrument manufacturers.

5.1 Carrier gases: use one of the following.

5.1.1 Carbon dioxide (CO_2), purity 99,9 % (volume fraction) for samples with moderate nitrogen contents; 99,995 % (volume fraction) for samples with low nitrogen contents.

5.1.2 Helium (He), purity 99,996 % (volume fraction).

5.2 Oxygen (O_2), purity 99,995 % (volume fraction).

5.3 Absorbent for SO_2, e.g. lead chromate to eliminate any sulfur compounds from the combustion products.

5.4 Copper oxide platinum catalyst (as filling material for the post-combustion tube).

Platinum catalyst (5 % of Pt on Al_2O_3) is blended with CuO at a ratio of 1 to 7, 8 or 9 parts.

To prevent separation as result of the different bulk densities of the two materials, it is not recommended to prepare the mixture before filling the tube. It is advisable to pour the platinum catalyst and copper oxide simultaneously into the post-combustion tube using a suitable funnel.

5.5 Silver wool.

Silver wool should be desegregated before being inserted in the post-combustion or reduction tube.

5.6 Silica (quartz) or **glass wool**.

5.7 Copper or **tungsten** (wire, cuttings or powder), suitable for filling the reduction tube.

To improve the precision of analytical results for low nitrogen contents, the use of copper or tungsten wire is recommended.

5.8 Phosphorus pentoxide (P_2O_5) or **granulated magnesium perchlorate** [$Mg(ClO_4)_2$], or another suitable support material, to fill the drying tubes.

5.9 Hollow corundum spheres or **aluminium oxide pellets**, suitable for filling the combustion tube.

5.10 Copper oxide (CuO), suitable as a filling material for the combustion tube.

5.11 Sodium hydroxide (NaOH), on a support material.

5.12 Standard nitrogen compounds, e.g. aspartic acid ($C_4H_7NO_4$), ethylenediaminetetraacetic acid ($C_{10}H_{16}N_2O_8$) (see annex C), or other suitable reference reagents with known constant nitrogen content (e.g. urea, tyrosine, phenylalanine); minimum assay should be 99 %.

For calibration purposes (see 9.4), use a standard nitrogen compound to prepare a standard series with a nitrogen content of 4 mg to 100 mg in equal steps of about 5 mg nitrogen. Prepare a second standard series with a nitrogen content of 10 mg to 200 mg in equal steps of about 10 mg nitrogen.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to 0,000 1 g.

6.2 Drying oven, capable of maintaining a temperature of between 105 °C and 110 °C.

6.3 Crucibles, e.g. made of stainless steel or ceramic, or **tin capsules**, suitable for the Dumas apparatus used.

NOTE 1 Several commercial instruments are provided with an automatic sampler.

NOTE 2 Some solid samples (e.g. powders) can be pressed to form pellets.

6.4 Dumas apparatus, with a thermal conductivity detector and a suitable device for signal integration.

Suitable types of Dumas apparatus available on the market operate according to the general scheme as given in Figure A.1, although they may be different in arrangement, both in composition and function.

Flow diagrams of two available instruments are shown as examples in annex B (Figures B.1 and B.2).

NOTE Instruments from the manufacturer Elementar Analysensysteme GmbH and LECO Instruments[1)] have been found to be suitable.

To avoid leaks, O-rings used for sealing shall be slightly lubricated prior to installation using a high-vacuum grease.

Experience has shown that it is important to clean all pieces of quartz and glassware carefully and to remove fingerprints from the tubes using a suitable solvent (e.g. acetone) before inserting them in the furnace.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

8.1 General

Prepare the test sample in such a way that a homogeneous sample is obtained, which is representative of the product.

The preparation of the test samples may be carried out as given in subclause 8.1 of ISO 6732:1985.

NOTE In the future, however, the preparation of the test sample could be done automatically.

8.2 Liquid test samples

For liquid samples, such as milk and whey, the following pre-drying procedure is recommended when the instruments make use of metal sample boats or crucibles.

Weigh, to the nearest 0,000 1 g, 0,5 g to 3,5 g (depending on the nitrogen content and the type of instrument) of the prepared test sample into a boat or crucible and dry for 1 h in an oven (6.2) set at between 105 °C and 110 °C. If a rack loaded with up to 50 test samples is placed in the oven (6.2), a longer drying time could be necessary. Check whether the contents of the boats or crucibles are dry before taking them out of the oven.

1) This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

9 Procedure

9.1 General

Since various Dumas instruments are available, different in design and handling, the operator shall carefully follow the instrument manufacturer's instructions for instrument setting up, optimization, calibration and operation. Switch the instrument on and allow its operating conditions to stabilize for about 1 h.

To assure that equivalent results are obtained**,** reference materials should be regularly used for method and instrument performance testing, as far as such materials are available. A matrix/instrument specific correction factor derived from those measurements may be used, if necessary.

If reference materials are not available, a regular comparison using the Kjeldahl method described in ISO 8968-1 | IDF 20-1 is recommended.

9.2 Test portion

Weigh, to the nearest 0,000 1 g, at least 0,2 g of the test sample (8.1 or 8.2) into a crucible or tin capsule (6.3). For samples with a protein content of less then 1 % (mass fraction), increase the amount of the test portion up to 3,5 g, depending on both the type of the Dumas equipment used and the nature of the test sample.

9.3 Control of oxygen demand

Some types of Dumas equipment require an estimation of the oxygen demand of the test portion. The calculated oxygen demand of some compounds used for calibration is given in annex C, Table C.1. For instruments with a self-optimizing oxygen control, the mass fraction of the residual oxygen content shall be between 2 % and 8 %.

9.4 Calibration

Pure standard nitrogen compounds (5.12) with a known constant nitrogen content are used for long-term instrument calibration. To prepare a calibration graph, the compound and its mass should be chosen in such a way that an absolute mass of nitrogen between 4 mg and 100 mg could be detected. For calibration, use 10 to 20 (or more) samples about equally covering the mass range of nitrogen. The same shall apply to the range between 10 mg to 200 mg of nitrogen. For more than 200 mg of nitrogen the calibration curve is expected to be nonlinear. In this nonlinear section, several straight segments may be used for the calibration. To assure the quality of calibration in this section, sample intakes should differ in steps of 1 mg to 5 mg of nitrogen.

Calibration may also be performed using aqueous standard solutions of nitrogen compounds.

Before starting a series of determinations, check the instrument response by running at least three nitrogen standards of known content. When the response is constant and the values obtained correspond to the long-term calibration as established above, proceed with the determination of the daily calibration factor by analysing at least four standard nitrogen compounds representing a nitrogen mass higher than the test samples to be analysed.

Use this factor for calibration of the actual measurement series.

Full range re-calibration will become necessary if the daily calibration factor deviates from its expected value by more than 10 % relative, or if essential parts of the instrument having a direct influence on calibration (e.g. thermal conductivity detector) have been replaced.

9.5 Determination

Operate the instrument and introduce the test portion according to manufacturer's instructions.

During analysis, the following processes take place in the instrument (see Figures B.1 and B.2):

The test portion is quantitatively combusted under standardized conditions. Volatile decomposition products (mainly N_2, NO_x, CO_2, H_2O) are transported by the carrier gas through a ballast column, if available on the instrument employed, or directly. The nitrogen oxides are reduced to molecular nitrogen and the excess of oxygen is bound in the reduction column.

Water is removed by means of an appropriate cooler, magnesium perchlorate or other drying agents. Unless carbon dioxide is used as carrier gas, it is removed by being passed over a suitable absorbent, e.g. sodium hydroxide on a supporting material.

Interfering compounds (e.g. volatile halogen and sulfur compounds) are removed by absorbents or contact materials (e.g. silver wool, sodium hydroxide on a suitable support material, or by all other material proposed by the manufacturers).

The nitrogen in the residual gas mixture consisting of nitrogen and carrier gas is passed through a thermal conductivity detector.

9.6 Detection and integration

A sensitive thermal conductivity cell, provided with automatic zero adjustment between each measurement of individual test portions and optimized for the carrier gas employed, is used for quantitative nitrogen determination. After amplification and A/D conversion of the detector signal**,** the obtained data are processed by a microprocessor and transferred to a personal computer.

10 Calculation and expression of results

10.1 Calculation

10.1.1 Nitrogen content

Results for the total nitrogen content, w_N, expressed as a percentage by mass, are available from the computer readouts or its printouts.

10.1.2 Crude protein content

The crude protein content, w_p, is obtained by using the following equation:

$$w_p = w_N \cdot R_f$$

where

w_p is the crude protein content, expressed as a percentage by mass;

w_N is the nitrogen content, expressed as a percentage by mass (10.1.1);

R_f is the agreed ratio factor (R_f = 6,38) between the protein and nitrogen content.

10.2 Expression of results

Express the results to three decimal places.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method have been published [7], [8].

The values for repeatability limit and reproducibility limit have been derived from the results of two interlaboratory tests carried out in accordance with ISO 5725-1. The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given.

NOTE 1 IDF 135 provides specific guidance for interlaboratory tests on methods of analysis and milk products. It is based on ISO 5725.

NOTE 2 The results of the first study (Study A) have been presented [7]. The results of the second study (Study B) have been published [8].

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than a mass fraction of:

a) Study A

- for UHT milk (3,5 % fat) with a nitrogen content of 0,565 %: 0,015 %,
- for dried skimmed milk with a nitrogen content of 5,767 %: 0,050 %;

b) Study B

- for fluid milk (2 % fat) with a nitrogen content of 0,489 %: 0,080 %,
- for fluid whey with a nitrogen content of 0,127 %: 0,035 %,
- for yogurt with a nitrogen content of 0,730 %: 0,080 %,
- for evaporated milk with a nitrogen content of 1,005 %: 0,022 %,
- for sweetened condensed milk with a nitrogen content of 1,113 %: 0,053 %,
- for cream cheese with a nitrogen content of 1,100 %: 0,133 %,
- for Cheddar cheese with a nitrogen content of 3,700 %: 0,093 %,
- for Swiss cheese with a nitrogen content of 4,478 %: 0,182 %,
- for Parmesan cheese with a nitrogen content of 6,395 %: 0,208 %,
- for non-fat dry milk with a nitrogen content of 5,500 %: 0,053 %,
- for whey protein concentrate with a nitrogen content of 5,614 %: 0,078 %,
- for sodium caseinate with a nitrogen content of 13,864 %: 0,031 %.

11.3 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than a mass fraction of:

a) Study A

- for UHT milk with 3,5 % fat and a nitrogen content of 0,565 %: 0,041 %,
- for dried skimmed milk with a nitrogen content of 5,767 %: 0,140 %;

b) Study B

- for fluid milk with 2 % fat and a nitrogen content of 0,489 %: 0,093 %,
- for fluid whey with a nitrogen content of 0,127 %: 0,080 %,
- for yogurt with a nitrogen content of 0,703 %: 0,111 %,
- for evaporated milk with a nitrogen content of 1,005 %: 0,089 %,
- for sweetened condensed milk with a nitrogen content of 1,113 %: 0,311 %,
- for cream cheese with a nitrogen content of 1,100 %: 0,182 %,
- for Cheddar cheeses with a nitrogen content of 3,700 %: 0,444 %,
- for Swiss cheese with a nitrogen content of 4,478 %: 0,324 %,
- for Parmesan cheese with a nitrogen content of 6,395 %: 0,226 %,
- for non-fat dry milk with a nitrogen content of 5,500 %: 0,169 %,
- for whey protein concentrate with a nitrogen content of 5,614 %: 0,244 %,
- for sodium caseinate with a nitrogen content of 13,684 %: 0,217 %.

12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incident which may have influenced the test result(s);
- the test result(s) obtained;
- if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Flowchart for the basic design of a Dumas apparatus

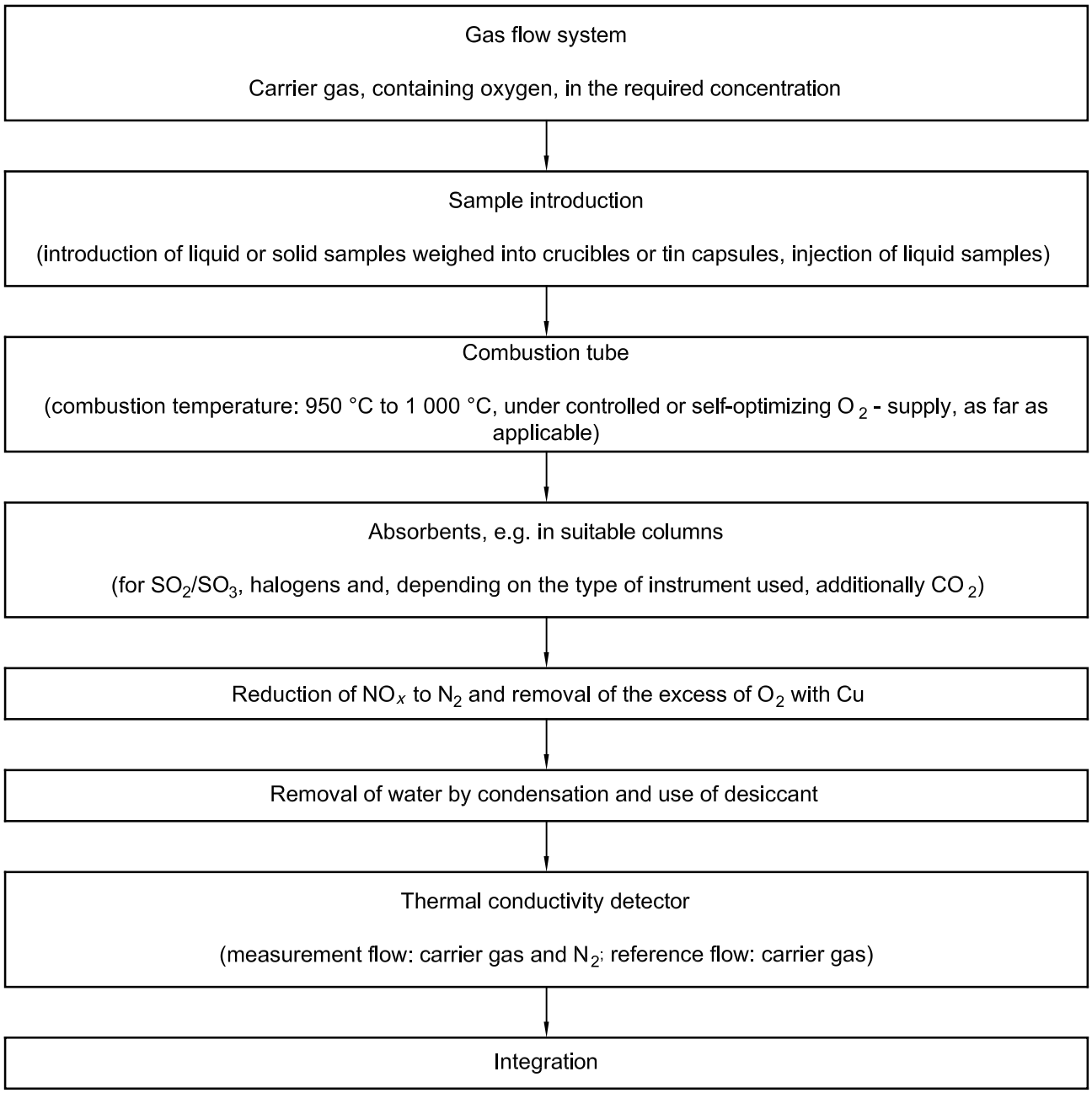

Figure A.1 — Simplified general scheme according to annexes B, C and D

Annex B
(informative)

Schemes of suitable types of Dumas apparatus

See Figures B.1 and B.2.

Key

1 Oxygen flow control
2 Sample loader
3 Resistance furnace with crucible
4 (Thermoelectric) cooler
5 Mixing container (ballast column)
6 Aliquot doser
7 Sodium hydroxide on support material
8 Magnesium perchlorate
9 Copper catalyst (reduces NO_x and O_2)
10 Thermal conductivity detector

[a] Surplus of combustion gases
[b] Measuring flow
[c] Reference flow

Figure B.1 — Diagram 1 of a Dumas apparatus (carrier gas He)

Key

drying agent

silver wool

copper wire

copper oxide with platinum catalyst

lead chromate

1 Test crucible
2 Combustion column
3 Combustion furnace (mobile)
4 Crucible holder
5 SO_2 absorption tube
6 Post-combustion tube
7 Reduction column
8 Drying tube
9 Thermal conductivity detector
10 Integrator

Figure B.2 — Diagram 2 of a Dumas apparatus

Annex C
(informative)

Equipment calibration

C.1 Calibration compounds

Table C.1 provides information additional to that in 9.3.

Some of the instruments available require entry of the expected oxygen demand.

Table C.1 — Oxygen demand of pure compounds suitable for calibration of the equipment

Compound	Nitrogen content % (mass fraction)	Maximum theoretical oxygen demand ml/g	Empirical oxygen demand ml/g
Urea	46,65	1 305	560
Aspartic acid	10,52	800	631
Tyrosine	7,73	1 391	1 270
Phenylalanine	8,48	1 593	1 460
Ethylenediamine-tetraacetic acid	9,59	920	769

C.2 Examples

C.2.1 General

The examples given below are for the calculation of the estimated oxygen demand.

NOTE These calculations are necessary for those types of instruments containing a measured oxygen supply (moderate O_2 surplus in the presence of CO_2 as carrier gas). All calculations proceed on the assumption that the samples consist only of the elements C, N, H and O.

C.2.2 Example 1

Urea (H_2NCONH_2): 1 mole corresponds to 60,06 g, sample mass 1 000 mg.

Therefore, 1 000 mg of urea contains:

199,8 mg of C;

66,6 mg of H;

466,5 mg of N;

266,4 mg of O.

The amount of oxygen required for complete combustion to carbon dioxide and water is calculated taking into account the oxygen content of the compound and the following:

— the molar volume of an ideal gas is 22,4 l;

— 1 mole of C corresponds to 12 g (12 000 mg);

— 1 mole of H_2 corresponds to 2 g (2 000 mg);

— 1 mole of N_2 corresponds to 28 g (28 000 mg);

— 1 mole of O_2 corresponds to 32 g (32 000 mg).

As result, 1 305 ml of oxygen is needed for combustion of 1 g of urea.

C.2.3 Example 2

Aspartic acid ($HO_2CCH_2CH(NH_2)CO_2H$): 1 mole corresponds to 133,10 g, sample mass 1 000 mg.

Therefore, 1 000 mg of aspartic acid contains:

360,6 mg of C;

52,6 mg of H;

105,2 mg of N;

480,8 mg of O.

The amount of oxygen required for complete combustion to carbon dioxide and water is calculated taking into account the oxygen content of the compound and the following facts:

— the molar volume of an ideal gas is 22,4 ml;

— 1 mole of C corresponds to 12 g (12 000 mg);

— 1 mole of H_2 corresponds to 2 g (2 000 mg);

— 1 mole of N_2 corresponds to 28 g (28 000 mg);

— 1 mole of O_2 corresponds to 32 g (32 000 mg).

As a result, 800 ml of oxygen are needed for combustion of 1 g of aspartic acid.

Annex D
(informative)

Comparison of results using the Dumas method with those obtained by the Kjeldahl method

Recent interlaboratory studies and several proficiency testing studies show that results for milk, which has not been predried, obtained using the Dumas method are not fully comparable to those obtained with the Kjeldahl method, as specified in ISO 8968-1 | IDF 20-1, and do not fulfil the precision limits of the latter method.

However, reported precision data for yogurt, evaporated milk, sweetened condensed milk, cream cheese, Cheddar cheese, Swiss cheese, Parmesan cheese, non-fat dry milk, whey protein concentrate, sodium caseinate and dried skimmed milk (under repeatability and reproducibility conditions) obtained using the Dumas method are equal or even better than those obtained with the Kjeldahl method for the same test sample [7], [8].

Mean values obtained using the Dumas method for test samples of milk and milk products (for raw and whole milk, skimmed milk powder, whey powder, whole milk powder, whey protein concentrate, cheese and whey) are generally 1 % to 3 % (relative) higher than those obtained with the Kjeldahl method [10], [11] for the same test sample.

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