SRI LANKA STANDARD 396 : 2012
ISO 3091 : 1975

METHODS OF TEST FOR
MEAT AND MEAT PRODUCTS
DETERMINATION OF NITRATE CONTENT
(First Revision)

SRI LANKA STANDARDS INSTITUTION

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Sri Lanka Standard
METHODS OF TEST FOR MEAT AND MEAT PRODUCTS
DETERMINATION OF NITRATE CONTENT
(First Revision)

NATIONAL FOREWORD

This Sri Lanka standard was approved by the Sectoral Committee on Agricultural and Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2012-01-27.

This standard was first published in 1976, which has been derived from the International Organization for Standardization on the subject of testing meat and meat products. This revision has been undertaken to update the standard to be in line with the available ISO standard for meat and meat products.

This standard is identical with ISO 3091 : 1975, Meat and meat products – Determination of nitrate content– Reference method, published by the International Organization for Standardization (ISO).

Terminology and Conventions:

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point on the base line as the decimal marker.

c) Wherever page numbers are quoted, they are ISO page numbers.

CROSS REFERENCE

International Standard	Corresponding Sri Lanka Standard
ISO 2918, Meat and meat products - Determination of nitrite content	SLS 384
ISO 3100, Meat and meat products – Sampling	No corresponding Sri Lanka standards

INTERNATIONAL STANDARD 3091

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

Meat and meat products — Determination of nitrate content (Reference method)

Viandes et produits à base de viande — Détermination de la teneur en nitrates (Méthode de référence)

First edition — 1975-09-01

UDC 637.5 : 546.175

Ref. No. ISO 3091-1975 (E)

Descriptors : meat, meat products, chemical analysis, determination of content, nitrates.

Price based on 5 pages

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 3091 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products,* and circulated to the Member Bodies in May 1974.

It has been approved by the Member Bodies of the following countries :

Australia	Germany	South Africa, Rep. of
Austria	Hungary	Spain
Bulgaria	India	Thailand
Czechoslovakia	Ireland	Turkey
Denmark	Israel	United Kingdom
Egypt, Arab Rep. of	Netherlands	U.S.S.R.
Ethiopia	Poland	Yugoslavia
France	Romania	

The Member Body of the following country expressed disapproval of the document on technical grounds :

Canada

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INTERNATIONAL STANDARD ISO 3091-1975 (E)

Meat and meat products — Determination of nitrate content (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the nitrate content of meat and meat products.

2 REFERENCES

ISO 2918, *Meat and meat products — Determination of nitrite content (Reference method).*

ISO 3100, *Meat and meat products — Sampling.*

3 DEFINITION

nitrate content of meat and meat products : The nitrate content determined according to the procedure described in this International Standard and expressed as milligrams of potassium nitrate per kilogram (parts per million).

4 PRINCIPLE

Extraction of a test portion with hot water, precipitation of the proteins and filtration.

Reduction of the extracted nitrates to nitrite by metallic cadmium. Development of a red colour by addition of sulphanilamide and *N*-1-naphthylethylenediamine dihydrochloride to the filtrate and photometric measurement at a wavelength of 538 nm.

5 REAGENTS

All reagents shall be of analytical quality. The water used shall be distilled water or water of at least equivalent purity.

5.1 Zinc rods, length about 15 cm and diameter 5 to 7 mm.

5.2 Solutions for precipitation of proteins

5.2.1 Reagent I

Dissolve 106 g of potassium ferrocyanide trihydrate $[K_4Fe(CN)_6 \cdot 3H_2O]$ in water and dilute to 1 000 ml.

5.2.2 Reagent II

Dissolve 220 g of zinc acetate dihydrate $[Zn(CH_3COO)_2 \cdot 2H_2O]$ and 30 ml of glacial acetic acid in water and dilute to 1 000 ml.

5.2.3 Borax solution, saturated

Dissolve 50 g of disodium tetraborate decahydrate $(Na_2B_4O_7 \cdot 10H_2O)$ in 1 000 ml of tepid water and cool to room temperature.

5.3 Cadmium sulphate solution, 30 g/l.

Dissolve 37 g of cadmium sulphate $(3CdSO_4 \cdot 8H_2O)$ in water and dilute to 1 000 ml.

5.4 Hydrochloric acid solution, about 0,1 N.

Dilute 8 ml of concentrated hydrochloric acid solution (ρ_{20} 1,19 g/ml) to 1 000 ml with water.

5.5 Ammonia buffer solution, pH 9,6 to 9,7.

Dilute 20 ml of concentrated hydrochloric acid (ρ_{20} 1,19 g/ml) with 500 ml of water. After mixing, add 10 g of ethylenediamine tetra-acetic acid disodium-salt dihydrate, $[CH_2N(CH_2COOH)CH_2COONa]_2 \cdot 2H_2O$, and 55 ml of concentrated ammonia (ρ_{20} 0,88 g/ml). Dilute to 1 000 ml with water and mix. Check the pH.

5.6 Sodium nitrite standard solutions.

Dissolve 1,000 g of sodium nitrite ($NaNO_2$) in water and dilute to 100 ml in a one-mark volumetric flask. Pipette 5 ml of the solution into a 1 000 ml one-mark volumetric flask. Dilute to the mark.

Prepare a series of standard solutions by pipetting 5 ml, 10 ml and 20 ml of this solution into 100 ml one-mark volumetric flasks and diluting to the mark with water. These standard solutions contain respectively 2,5 μg, 5,0 μg and 10,0 μg of sodium nitrite per millilitre.

The standard solutions and the dilute (0,05 g/l) sodium nitrite solution from which they are prepared shall be made up on the day of use.

5.7 Solutions necessary for colour development

5.7.1 Solution I

Dissolve, by heating on a water bath, 2 g of sulphanilamide ($NH_2C_6H_4SO_2NH_2$) in 800 ml of water. Cool, filter, if necessary, and add 100 ml of concentrated hydrochloric acid solution (ρ_{20} 1,19 g/ml), while stirring. Dilute to 1 000 ml with water.

5.7.2 Solution II

Dissolve 0,25 g of *N*-1-naphthylethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) in water. Dilute to 250 ml with water.

Store the solution in a well-stoppered brown bottle. It shall be kept in a refrigerator, for not longer than one week.

5.7.3 Solution III

Dilute 445 ml of concentrated hydrochloric acid solution (ρ_{20} 1,19 g/ml) to 1 000 ml with water.

5.8 Potassium nitrate standard solution.

Dissolve 1,465 g of potassium nitrate (KNO_3) in water and dilute to 100 ml in a one-mark volumetric flask. Pipette 5 ml of the solution into a 1 000 ml volumetric flask and dilute to the mark.

This solution contains 73,25 µg/ml of potassium nitrate.

This standard solution shall be prepared on the day of use.

6 APPARATUS

Usual laboratory equipment and the following items :

6.1 Mechanical meat mincer, laboratory size, fitted with a perforated plate with holes not greater than 4 mm in diameter.

6.2 Analytical balance.

6.3 One-mark volumetric flasks of 100 ml, 200 ml and 1 000 ml, complying with ISO/R 1042, Class B.

6.4 One-mark pipettes of 10 ml and 20 ml and, if necessary, with another capacity, according to the aliquot of filtrate (8.8.1), complying with ISO/R 648, Class A.

6.5 Boiling water bath.

6.6 Fluted filter paper, diameter about 15 cm, free of nitrite and nitrate.

6.7 Glass equipment for the reduction of the nitrate (see figure).

6.8 Photoelectric colorimeter or **spectrophotometer** with cells of 1 cm optical path length.

6.9 Conical flask, 300 ml.

7 SAMPLE

7.1 Proceed from a representative sample of at least 200 g. See ISO 3100.

7.2 Prepare the test sample (8.1) immediately or, if this cannot be done, store the sample at a temperature of 0 to 5 °C, for not longer than 4 days.

8 PROCEDURE

8.1 Preparation of test sample

Make the sample homogeneous by passing it at least twice through the meat mincer (6.1) and mixing. Keep it in a completely filled, air-tight, closed container under refrigeration.

Analyse the test sample as soon as possible, but always within 24 h.

NOTE – In the case of uncooked products, analyse immediately after homogenization.

8.2 Preparation of the cadmium column

8.2.1 Place 3 to 5 zinc rods (5.1) in the cadmium sulphate solution (5.3) contained in a beaker (1 l of cadmium sulphate solution is sufficient for preparing one cadmium column).

8.2.2 Remove the spongy metallic cadmium deposit from the zinc rods every 1 or 2 h by swirling them in the solution or rubbing them against each other.

8.2.3 Finally, after 6 to 8 h, decant the solution and wash the deposit twice with 1 l of water, taking care that the cadmium is continuously covered with a layer of liquid.

8.2.4 Transfer the cadmium deposit with 400 ml of hydrochloric acid solution (5.4) to a laboratory mixer and blend for 10 s.

Return the contents of the mixer to the beaker.

8.2.5 Occasionally stir up the cadmium deposit with a glass rod. After leaving it for a night under hydrochloric acid solution, stir once more to remove all bubbles of gas from the cadmium.

8.2.6 Decant the solution and wash the cadmium slurry twice, each time with 1 l of water.

8.2.7 Fit a glass wool plug to the bottom of the glass column intended to contain the cadmium (see figure).

8.2.8 Wash the cadmium into the glass column with water until the height of the cadmium bed is about 17 cm. Drain the column occasionally during filling, taking care not to allow the level of the liquid to fall below the top of the cadmium bed. Eliminate inclusions of gas (for example with a knitting needle). The liquid should flow out at a rate not exceeding 3 ml/min.

8.3 Test portion

Weigh, to the nearest 0,001 g, 10 g of the test sample.

8.4 Deproteination

8.4.1 Transfer the test portion quantitatively into the conical flask (6.9) and add successively 5 ml of saturated borax solution (5.2.3) and 100 ml of water at a temperature not below 70 °C.

8.4.2 Heat the flask and its contents for 15 min on the boiling water bath (6.5) and shake repeatedly.

8.4.3 Allow the flask and its contents to cool to room temperature and add successively 2 ml of reagent I (5.2.1) and 2 ml of reagent II (5.2.2). Mix thoroughly after each addition.

8.4.4 Transfer the contents to a 200 ml one-mark volumetric flask (6.3). Dilute to the mark with water and mix. Allow the flask to stand for 30 min at room temperature.

8.4.5 Carefully decant the supernatant liquid and filter it through the fluted filter paper (6.6) so as to obtain a clear solution.

NOTE – If it is required to determine both the nitrate and the nitrite content on the same sample, the same deproteinated filtrate can be used for both.

8.5 Pre-treatment of the cadmium column

Wash the cadmium column successively with 25 ml of hydrochloric acid solution (5.4), 50 ml of water, and 25 ml of the 1 + 9 diluted ammonia buffer solution (5.5). Do not permit the level of the liquid in the funnel to fall below the top of the capillary inlet tube of the cadmium column.

8.6 Checking the reducing capacity of the cadmium column

8.6.1 Pipette 20 ml of potassium nitrate standard solution (5.8) and simultaneously add 5 ml of ammonia buffer solution (5.5), into the reservoir on top of the cadmium column. Collect the effluent in a 100 ml one-mark volumetric flask (6.3).

8.6.2 When the reservoir is nearly empty, wash the walls with about 15 ml of water; repeat the same treatment with another 15 ml portion of water.

After this portion has run into the column as well, completely fill the reservoir with water.

8.6.3 After nearly 100 ml of effluent has been collected, remove the flask from under the column and dilute to the mark with water.

8.6.4 Pipette 10 ml of the eluate into a 100 ml one-mark volumetric flask (6.3) and proceed as specified in 8.8.2 to 8.8.4.

8.6.5 If the nitrite concentration of the eluate, as determined from the calibration curve (see 8.10), is below 0,9 μg of sodium nitrite per millilitre (i.e. 90 % of theoretical value), the cadmium column should be rejected.

8.7 Reduction of nitrate to nitrite

8.7.1 Pipette into the reservoir on top of the column 20 ml of the filtrate (8.4.5) and simultaneously add 5 ml of ammonia buffer solution (5.5).

Collect the effluent from the column in a 100 ml one-mark volumetric flask (6.3).

8.7.2 Proceed as specified in 8.6.2 and 8.6.3.

8.8 Colour measurement

8.8.1 Pipette an aliquot portion of the eluate (V ml), but not more than 25 ml, into a 100 ml one-mark volumetric flask (6.3) and add water to obtain a volume of about 60 ml.

8.8.2 Add 10 ml of solution I (5.7.1), followed by 6 ml of solution III (5.7.3), mix and leave the solution for 5 min at room temperature in the dark.

8.8.3 Add 2 ml of solution II (5.7.2), mix and leave the solution for 3 to 10 min at room temperature in the dark. Dilute to the mark with water.

8.8.4 Measure the absorbance of the solution in a 1 cm cell using a photoelectric colorimeter or a spectrophotometer (6.8) at a wavelength of about 538 nm.

NOTE – If the absorbance of the coloured solution obtained from the test portion exceeds that obtained for the standard solution with the highest concentration, repeat the operations described in 8.8, reducing the quantity of eluate pipetted in 8.8.1.

8.9 Number of determinations

Carry out two independent determinations, beginning with different test portions taken from the same test sample.

8.10 Calibration curve

8.10.1 Pipette respectively into four 100 ml one-mark volumetric flasks (6.3) 10 ml of water and 10 ml of each of the three sodium nitrite standard solutions (5.6), containing 2,5 μg, 5,0 μg and 10,0 μg of nitrite per millilitre.

8.10.2 To each flask add water to obtain a volume of about 60 ml and proceed as described in 8.8.2 to 8.8.4.

8.10.3 Draw the calibration curve by plotting the measured absorbances against the concentrations, in micrograms per millilitre, of the standard sodium nitrite solutions.

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

Calculate the nitrate content of the sample, expressed as milligrams of potassium nitrate per kilogram, using the formula :

$$KNO_3 = 1{,}465 \left(c \times \frac{10\,000}{m \times V} - NaNO_2 \right)$$

where

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

V is the volume, in millilitres, of the aliquot portion of the eluate (see 8.8.1);

c is the concentration of sodium nitrite, in micrograms per millilitre, read from the calibration curve, that corresponds with the absorbance of the solution prepared from the test portion (see 8.8.4);

$NaNO_2$ is the nitrite content of the sample, expressed as milligrams of sodium nitrite per kilogram and determined according to ISO 2918.

Take as the result the arithmetic mean of the two determinations, provided that the requirement for repeatability (see 9.2) is satisfied. Express the result to the nearest 1 mg per kilogram of product.

9.2 Repeatability

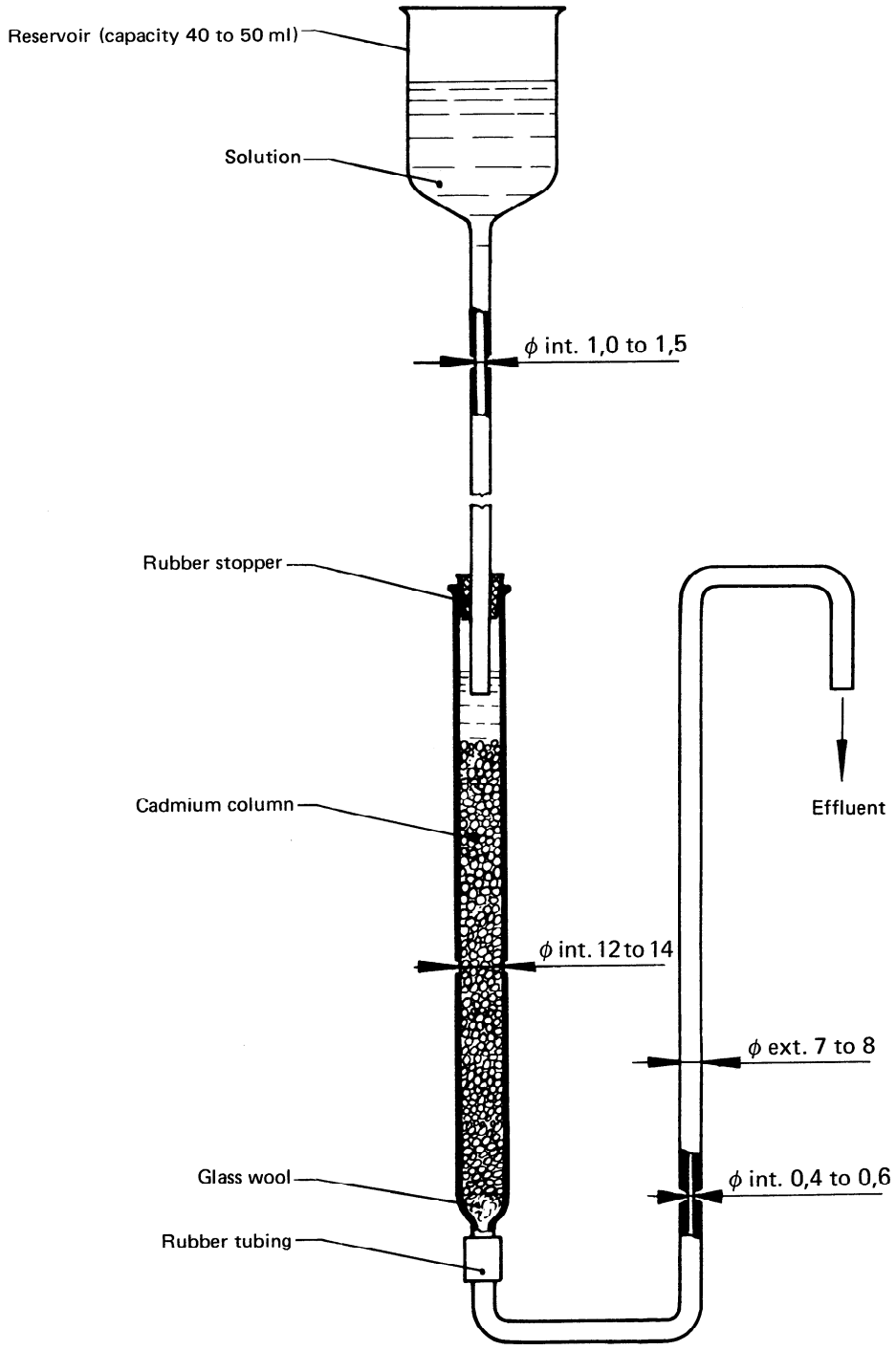
The difference between the results of two determinations carried out simultaneously or in rapid succession, by the same analyst, shall not be greater than 10 % of the mean value.

10 TEST REPORT

The test report shall show the method used and the result obtained; it shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

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

Dimensions in millimetres



NOTE – A flexible connection may be used between the bottom of the column and the effluent capillary tube, in order to allow adjustment of the height of the capillary tube and thus of the flow rate.

FIGURE – **Apparatus for nitrate reduction**

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SRI LANKA STANDARDS INSTITUTION

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