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.

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

Canada

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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 $[K_4Fe(CN)_6.3H_2O]$ in water and dilute to 1 000 ml.

5.2.2 Reagent II

Dissolve 220 g of zinc acetate dihydrate $[Zn(CH_3COO)_2.2H_2O]$ 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 $(Na_2B_4O_7.10H_2O)$ 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 $(3CdSO_4.8H_2O)$ 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, $[CH_2N(CH_2COOH)CH_2COONa]_2.2H_2O$, 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.

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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).

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