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

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FOREWORD

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International Standard ISO 2918 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products,* and circulated to the Member Bodies in April 1974.

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

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

1 SCOPE AND FIELD OF APPLICATION

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

2 REFERENCE

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

3 DEFINITION

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

4 PRINCIPLE

Extraction of a test portion with hot water, precipitation of the proteins and filtration. In the presence of nitrite, development of a red colour by the 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 Solutions for precipitation of proteins

5.1.1 Reagent I

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

5.1.2 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.

5.1.3 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.

5.2 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.

5.3 Solutions necessary for colour development

5.3.1 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.

5.3.2 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.

5.3.3 Solution III

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

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, if necessary, with another capacity according to the aliquot of filtrate (8.4.1), complying with ISO/R 648, Class A.

6.5 Boiling water bath.

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

6.7 Fluted filter paper, diameter about 15 cm, free of nitrite.

6.8 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 Test portion

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

8.3 Deproteination

8.3.1 Transfer the test portion quantitatively into the conical flask (6.8) and add successively 5 ml of saturated borax solution (5.1.3) and 100 ml of water at a temperature not below 70 °C.

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

8.3.3 Allow the flask and its contents to cool to room temperature and add successively 2 ml of reagent I (5.1.1) and 2 ml of reagent II (5.1.2). Mix thoroughly after each addition.

8.3.4 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.

8.3.5 Carefully decant the supernatant liquid and filter it through the fluted filter paper (6.7) so as to obtain a clear solution.

8.4 Colour measurement

8.4.1 Pipette an aliquot portion of the filtrate (*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.

8.4.2 Add 10 ml of solution I (5.3.1), followed by 6 ml of solution III (5.3.3), mix and leave the solution for 5 min at room temperature, in the dark.

8.4.3 Add 2 ml of solution II (5.3.2), mix and leave the solution for 3 to 10 min at room temperature in the dark. Dilute to the mark with water.

8.4.4 Measure the absorbance of the solution in a 1 cm cell using a photoelectric colorimeter or a spectrophotometer (6.6), 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 8.4, reducing the quantity of filtrate pipetted in 8.4.1.

8.5 Number of determinations

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

8.6 Calibration curve

8.6.1 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 (5.2), containing 2,5 µg, 5,0 µg and 10,0 µg of nitrite per millilitre.

8.6.2 To each flask add water to obtain a volume of about 60 ml and proceed as described in 8.4.2 to 8.4.4.

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

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

Calculate the nitrite content of the sample, expressed as milligrams of sodium nitrite per kilogram, using the formula :

$$NaNO_2 = c \times \frac{2\ 000}{m \times V}$$

where

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

V is the volume, in millilitres, of the aliquot portion of the filtrate (see 8.4.1) taken for the photometric determination;

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 8.4.4).

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.

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