SLOVENSKI STANDARD
SIST EN 12014-3:2005

01-september-2005

Nadomešča:
SIST ENV 12014-3:1999

Živila - Določevanje nitratov in/ali nitritov - 3. del: Spektrometrijsko določevanje nitratov in nitritov v mesnih proizvodih po encimski redukciji nitratov v nitrite

Foodstuffs - Determination of nitrate and/or nitrite content - Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite

Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 3: Spektralphotometrische Bestimmung des Nitrat- und Nitritgehaltes in Fleischerzeugnissen nach enzymatischer Reduktion von Nitrat zu Nitrit

Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites - Partie 3 : Détermination spectrométrique de la teneur en nitrates et en nitrites des produits carnés apres réduction enzymatique des nitrates en nitrites

Ta slovenski standard je istoveten z: **EN 12014-3:2005**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.120.10	Meso in mesni proizvodi	Meat and meat products

SIST EN 12014-3:2005 **en**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 12014-3

May 2005

ICS 67.120.10

Supersedes ENV 12014-3:1998

English version

Foodstuffs - Determination of nitrate and/or nitrite content - Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite

Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites des produits carnés - Partie 3: Détermination spectrométrique de la teneur en nitrates et en nitrites après réduction enzymatique des nitrates en nitrites

Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 3: Spektralphotometrische Bestimmung des Nitrat- und Nitritgehaltes in Fleischerzeugnissen nach enzymatischer Reduktion von Nitrat zu Nitrit

This European Standard was approved by CEN on 1 April 2005.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 12014-3:2005: E

Contents

Foreword

This European Standard (EN 12014-3:2005) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2005, and conflicting national standards shall be withdrawn at the latest by November 2005.

This document supersedes ENV 12014-3:1998.

This series *Foodstuffs - Determination of nitrate and/or nitrite content* consist of the following parts:

Part 1: General considerations;

Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products;

Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite;

Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products;

Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants;

Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after Cadmium reduction.

This European Standard includes a Bibliography.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This European Standard specifies a spectrometric method for the determination of nitrate and nitrite content of meat products and has been validated for different meat products with a content of 9 mg/kg to 22 mg/kg nitrite calculated as sodium nitrite and 23 mg/kg to 48 mg/kg nitrate calculated as sodium nitrate.

NOTE Experiences have shown that the method is also applicable for total nitrite and nitrate content from 5 mg/kg up to 125 mg/kg calculated as sodium nitrite. For further information on applicability, see [1].

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987).*

3 Principle

Nitrite in an aqueous extract of the test sample is treated with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride. The formed red compound is measured spectrometrically at a wavelength of 540 nm [2].

Nitrate in an aqueous extract of the analytical sample is converted into nitrite by nitrate reductase. This converted nitrite together with the nitrite which is already in the analytical sample reacts with sulfanilamide and N-(1-naphthyl)ethylenendiamine dihydrocloride. The colour intensity of this resulting red compound is measured in a spectrometer at 540 nm. The nitrate content is calculated from the difference between the spectrometric measurements.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents and materials of recognized analytical grade and water of at least grade 3 according to EN ISO 3696. When preparing solutions, the purities of the reagents available shall be taken into account.

4.2 Sodium hydroxide solution $c(NaOH) \approx 1$ mol/l[1)]

4.3 Carrez solution No. 1

Dissolve 150 g of potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \cdot 3\ H_2O$ in water and dilute to 1 000 ml. Store the solution in a brown bottle and replace it every two months.

4.4 Carrez solution No. 2

Dissolve 230 g of zinc acetate, $Zn\ (CH_3COO)_2 \cdot 2\ H_2O$, in water and dilute to 1 000 ml.

1) *c* is the substance concentration

4.5 Reagents for the enzymatic determination of nitrate[2)]

4.5.1 Imidazole buffer solution, pH = 7,8

Dissolve 0,68 g of imidazole in an 100 ml volumetric flask in 80 ml of water, adjust the pH to 7,8 with hydrochloric acid (c(HCl) = 2 mol/l) and dilute to the mark with water.

4.5.2 Solution of tetrasodium salt of reduced ß-nicotinamide adenine dinucleotide phosphate (ß-NADPH-Na_4, mass fraction of at least 98 %)

Dissolve 125 mg of reduced ß-nicotinamide adenine dinucleotide phosphate and 2,5 mg of disodium salt of flavin adenin dinucleotide (mass fraction of at least 88 %) in 50 ml of the imidazole buffer solution (4.5.1). Prepare this solution immediately before use.

4.5.3 Nitrate reductase buffer solution

Dissolve 11 mg of nitrate reductase lyophilisate from aspergillus sp. (EC 1.6.6.2, approximately 0,4 U/mg[3)]) in 10 ml of imidazole buffer solution (4.5.1) and add 50 mg of ethylenedintrilotetraacetic acid disodium salt-dihydrate.

The solution will be stable for about 14 days if stored at 4 °C.

4.6 Colour reagents

4.6.1 Colour reagent No. 1

Dissolve 8 g of sulfanilamide in 500 ml of water while heating on a water bath (5.3). Cool the solution to room temperature and filter if necessary. Add 330 ml of hydrochloric acid (4.9) while stirring continuously and dilute the solution to 1 000 ml with water. The solution will be stable for several weeks at room temperature.

4.6.2 Colour reagent No. 2

Dissolve 0,330 g of N-(1-naphthyl)ethylenediamine dihydrochloride in 250 ml of water. Keep the solution in a brown bottle and replace it every week.

4.6.3 Colour reagent mixture

Prepare the required amount of colour reagent mixture by mixing equal parts by volume of colour reagents No. 1 (4.6.1) and No. 2 (4.6.2) on the day of use.

4.7 Sodium nitrite solutions

4.7.1 General

It is recommended to prepare sodium nitrite standard solutions of sodium nitrite on the day of use.

---

2) The ‘Nitrat’ test kit for the enzymatic determination of nitrate and nitrite is the trade name of a product produced by Boehringer Mannheim/Roche Diagnostics, Mannheim, supplied by r-biopharm, Darmstadt (Germany) This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3) U, this unit (often called the International unit or standard unit) is defined as the amount of enzyme which catalyses the transformation of 1 µmol substrate per minute under standard conditions.

4.7.2 Sodium nitrite stock solution, $\rho(NaNO_2)$ = 400 mg/l

Dissolve 200 mg of sodium nitrite weighed to the nearest 0,1 mg in water in a 500 ml volumetric flask and dilute the solution to the mark with water.

The stock solution may be used for 2 weeks if stored in a refrigerator at 4 °C.

4.7.3 Diluted sodium nitrite stock solution $\rho(NaNO_2)$ = 20 mg/l

Pipette 25 ml of the sodium nitrite stock solution (4.7.2) into a 500 ml volumetric flask and dilute to the mark with water.

4.7.4 Sodium nitrite standard solutions

Pipette 10 ml, 20 ml, 30 ml and 40 ml, respectively, of the diluted stock solution (4.7.3) containing 0,13 mg, 0,27 mg, 0,40 mg, and 0,53 mg, respectively, of nitrite into a series of 200 ml volumetric flasks, add sodium hydroxide solution (4.2) to adjust the pH-value at 8,0 to 8,5 by using a pH-meter (5.5).

Add 4 ml each of Carrez solution No. 1 (4.3) and No. 2 (4.4), shaking after each addition, dilute to the mark with water, thoroughly mix the contents of the flask and filter through a fluted filter paper (5.4). Discard the first 20 ml of each of the filtrates.

4.8 Potassium nitrate solutions

4.8.1 General

It is recommended to prepare potassium nitrate solutions on the day of use.

4.8.2 Potassium nitrate stock solution, $\rho(KNO_3)$ = 600 mg/l[4)]

Dissolve 300 mg of potassium nitrate weighed to the nearest 0,1 mg in water in a 500 ml volumetric flask and dilute the solution to the mark with water.

4.8.3 Diluted potassium nitrate stock solution, $\rho(KNO_3)$ = 30 mg/l

Pipette 25 ml of the potassium nitrate stock solution (4.8.2) into a 500 ml volumetric flask and dilute to the mark with water.

4.8.4 Potassium nitrate standard solutions

Pipette 10 ml, 20 ml, 30 ml and 40 ml, respectively, of the diluted stock solution (4.8.3), corresponding to 0,14 mg, 0,27 mg, 0,41 mg, and 0,55 mg, respectively, of nitrite ion into a series of 200 ml volumetric flasks, add sodium hydroxide solution (4.2) to adjust the pH-value at 8,0 to 8,5 by using a pH-meter (5.5).

Add 4 ml each of Carrez solution No. 1 (4.3) and No. 2 (4.4), shaking after each addition, dilute to the mark with water, thoroughly mix the contents of the flask and filter through a fluted filter paper (5.4). Discard the first 20 ml of each of the filtrates.

4.9 Hydrochloric acid, fuming, w = 37 %[5)] , nitrite free

4) ρ is the mass concentration

5) w is the mass fraction

5 Apparatus and equipment

5.1 General

Usual laboratory apparatus and, in particular, the following:

5.2 Homogenizing equipment, mechanical or electrical, capable of homogenizing the test sample, this includes a high-speed rotational cutter, or a mincer fitted with a plate with holes not exceeding 4,5 mm in diameter.

5.3 Water bath, capable of being maintained at 100 °C

5.4 Fluted filter papers, nitrate and nitrite free

5.5 pH meter, with pH measuring cell (glass and reference electrodes)

5.6 Spectrometer, for carrying out measurements at a wavelength of 540 nm, with suitable cells, made of glass or plastics which have no significant optical absorption at a wavelength of 540 nm.

6 Procedure

6.1 Preparation of the sample solution

Homogenize the laboratory sample with the appropriate equipment (5.2) Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment. Weigh, to the nearest 10 mg, 10 g of the homogenized sample into e.g. a wide neck conical flask, add about 50 ml of water and homogenize for 30 s to 60 s. Rinse the shaft of the homogenizer into the flask with 50 ml of hot water. Add sodium hydroxide solution (4.2) to adjust the pH-value to 8,0 to 8,5 by using a pH-meter (5.5). Heat the flask for 15 minutes in a boiling water bath (5.3), shaking several times.

NOTE For uncooked meat products the pH should not exceed 8,5 since otherwise it may not be possible to clarify the solutions by filtering after adding the Carrez reagents. In the case of frankfurter type sausages or cooked samples like liver sausage, the clarification with Carrez is easier, and the pH may be allowed to rise to about 9,5.

Cool to room temperature and transfer the contents of the flask quantitatively to a 200 ml volumetric flask and add 4 ml each of Carrez solutions No. 1 (4.3) and No. 2 (4.4), shaking after each addition. Then dilute to the mark with water, mix thoroughly and filter through a fluted filter paper (5.4), discarding the first 20 ml of the filtrate. The clear residual filtrate is used for the determination (sample solution).

6.2 Preparation of the calibration graphs

6.2.1 Calibration graph for the nitrite content

Mix 2,0 ml of each of the sodium nitrite standard solutions (4.7.4) with 1,0 ml of water and 3,0 ml of the colour reagent mixture (4.6.3) in a test tube, shake and store the solution in the dark at room temperature.

After 30 min measure the absorbance values of each solution at a wavelength of 540 nm in a spectrometer (5.6) against water.

Plot the absorbance values obtained for the four sodium nitrite solutions (4.7.4) against the corresponding amounts of nitrite-ions (milligrams in 200 ml solution).