SLOVENSKI STANDARD
SIST EN 12014-7:1999

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Živila - Določevanje vsebnosti nitratov in/ali nitritov - 7. del: Kontinuirana pretočna metoda za določevanje vsebnosti nitratov v zelenjavi in zelenjavnih proizvodih po redukciji s kadmijem

Foodstuffs - Determination of nitrate and/or nitrite content - Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after Cadmium reduction

Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 7: Kontinuierliches Durchflußverfahren zur Bestimmung des Nitratgehaltes in Gemüse und Gemüseerzeugnissen nach Cadmiumreduktion

Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites - Partie 7: Détermination de la teneur en nitrates par flux continu dans les légumes et les produits a base de légumes, apres réduction au cadmium

Ta slovenski standard je istoveten z: **EN 12014-7:1998**

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.080.20	Zelenjava in zelenjavni proizvodi	Vegetables and derived products

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English version

Foodstuffs - Determination of nitrate and/or nitrite content - Part 7: Continuous Flow method for the determination of nitrate content of vegetables and vegetable products after Cadmium reduction

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Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 7: Kontinuierliches Durchflußverfahren zur Bestimmung des Nitratgehaltes in Gemüse und Gemüseerzeugnissen nach Cadmiumreduktion

This European Standard was approved by CEN on 13 May 1998.

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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.

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

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Content

Foreword

This European Standard 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 December 1998, and conflicting national standards shall be withdrawn at the latest by December 1998.

This series "Foodstuffs - Determination of nitrate and/or nitrite content" consists 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.

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

1 Scope

This European Standard specifies a continuous flow method (CF-method) for the determination of nitrate content of vegetables and vegetable products having a nitrate content of 900 mg/kg to 5200 mg/kg (calculated as nitrate ion).

NOTE: Experiences have shown that the method may also applied for vegetables and vegetable products having a nitrate content of greater than 50 mg/kg (calculated as nitrate ion).

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 12014-1
Foodstuffs - Determination of nitrate and/or nitrite content - Part 1: General considerations

EN ISO 3696
Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)

3 Principle

Test portions are extracted with water and filtered. The filtrate is transferred to the dializer of the continuous flow (CF) system [1]. An aliquot portion of the nitrate ions diffuses in the dializing unit with a hydrophilic membrane into a slightly alkaline buffer solution in which the nitrates are reduced to nitrite by metallic cadmium. The nitrite ions react with sulfanilamide and N-1-naphthylethylenediamine to give a reddish-purple azo dye.

The absorbance of this dye is determined spectrometrically at a wavelength between 520 nm and 540 nm, preferably at its maximum.

NOTE: The CF method is an automated version of the manual procedure for nitrate determinations in leafy vegetables as prescribed by the Official Dutch Food Act [2]. With the automated method the cadmium reductor may be used for a longer period of time without any appreciable loss of its reducing capacity. Also ready-made cadmium columns from commercial suppliers are available, minimizing the main objection of working with this toxic element.

4 Reagents

All reagents and materials used shall be of recognized analytical grade and water shall be of at least grade 1 according to EN ISO 3696. Consult safety data sheets or labels for additional information on toxicity, flammability and explosivity of chemicals used. When preparing solutions, the purities of the reagents available shall be taken into account.

4.1 Cadmium column, activated, ready-to-use, as commercially available.

CAUTION: Cadmium is extremely toxic for humans and the environment; take effective precautions before handling and disposing this compound.

Cadmium columns may also be prepared in the laboratory according to the description given in the annex A

4.2 Hydrochloric acid, concentrated, ρ_{20} (HCl) = 1,18 g/l

4.3 Hydrochloric acid c (HCl) = 4 mol/l [1])

Dilute 320 ml of hydrochloric acid (4.2) to 1 l with water.

4.4 Hydrochloric acid c (HCl) = 0,1 mol/l

4.5 Ammonia, $w(NH_3)$ = 25 %[2])

4.6 Ammonia, $w(NH_3)$ = 5 %

4.7 Ammonium chloride, NH_4Cl

[1]) c is the substance concentration
[2]) w is the mass fraction

4.8 Wetting agent, e.g. Polyethyleneglycol-mono [p-(1,1,3,3-tetramethylbutyl)-phenyl]-ether[3]) or polyethylene glycol ether.

4.9 Wetting agent solution

Dissolve 25 g of wetting agent (4.8) into 75 ml of methanol and then add 150 ml of water.

4.10 Phosphoric acid $w(H_3PO_4)$ = 85 %

4.11 Sulfanilamide ($NH_2C_6H_4SO_2NH_2$)

4.12 N-(1-naphthyl)ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2\ HCl$)

4.13 Ethylenediamine tetracetic (EDTA) acid disodium salt dihydrate
($[CH_2N(CH_2COOH)\ (CH_2COONa)]_2 \cdot 2\ H_2O$)

4.14 Copper sulfate solution, $\rho\ (CuSO_4 \cdot 5\ H_2O)$ = 2 g/100 ml [4])

4.15 Extracting buffer, pH 9,6 to 9,7

Add 50 ml of hydrochloric acid (4.2) to approximately 600 ml water and mix. Add 75 ml of ammonia (4.5), dilute to 1 l with water and mix. Adjust to a pH of 9,6 to 9,7 with either hydrochloric acid (4.3 or 4.4) or ammonia (4.5 or 4.6).

4.16 Buffer solution continuous flow

Dissolve 25 g of ammonium chloride (4.7) in 1 l of water. Add about 5 ml of wetting agent solution (4.9) and adjust to a pH of 9,0 with ammonia (4.5 or 4.6).

4.17 Diluting solution continuous flow

Add about 5 ml of 10 % wetting agent solution (4.9) to 1 l of water.

4.18 Colour reagent continuous flow

Add 50 ml of phosphoric acid (4.10) and 5 g of sulfanilamide (4.11) to 250 ml of water. Dissolve completely and heat, if necessary. Add 0,25 g of N-(1-naphthyl)ethylenediamine dihydrochloride (4.12) and dilute to 500 ml with water. Store at 4 °C in a well-stoppered bottle in the dark. This solution may be kept for up to 2 weeks but it should preferably be prepared daily.

4.19 Nitrate stock solution, $\rho\ (NO_3^-)$ = 2 g/l

Dissolve 0,815 g of dry potassium nitrate in water and dilute with water to 250 ml in a volumetric flask. Mix well. This stock solution is stable for at least 2 months if stored at 4 °C.

4.20 Nitrate standard solutions, $\rho\ (NO_3^-)$ = 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l and 600 mg/l

Pipette 2,5 ml, 5 ml, 10 ml, 15 ml, 20 ml, 25 ml and 30 ml of the nitrate stock solution (4.19) in seven subsequent 100 ml volumetric flasks. Dilute to the mark with water. Mix well. Prepare these standard solutions on the day of use.

4.21 Nitrite stock solution, $\rho\ (NO_2^-)$ = 1 g/l

Sodium nitrite is a hygroscopic substance. Dissolve 0,150 g of dry sodium nitrite in water and dilute to 100 ml in a volumetric flask. Mix well. This stock solution is stable for at least 2 weeks if stored at 4 °C.

4.22 Reducing capacity control solutions, $\rho\ (NO_2^-)$ = 100 mg/l, 200 mg/l and 300 mg/l

Pipette 5 ml, 10 ml and 15 ml of the nitrite stock solution (4.21) in three subsequent 50 ml volumetric flasks. Dilute to the mark with water. Mix well. Prepare these control solutions on the day of use.

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[3]) Polyethyleneglycol-mono [p-(1,1,3,3-tetramethylbutyl)-phenyl]-ether is commercially available as Triton X®-100 (pro analyse). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

[4]) ρ is the mass concentration

4.23 Conditioner solution, ρ (NO_3^-) = 10 mg/l

Dilute 5 ml of the nitrate stock solution (4.19) to 1 l with water. Mix well. Prepare on the day of use.

4.24 EDTA solution, ρ (EDTA) = 34 g/l

Dissolve 3,4 g of the disodium dihydrate salt of EDTA (4.13) in 100 ml of water.

4.25 Regeneration solution

Add about 5 ml of EDTA solution (4.24) and 2 ml of HCl solution (4.4) to 100 ml of water.

4.26 Hypochlorite solution *w* (HOCl) = 0,5%

Prepare by diluting a commercially available 5 % solution.

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

5.1 Laboratory cutter

5.2 Balance or Gravimetric dilutor

5.3 Homogenizer

5.4 Continuous Flow System, consisting of sampler, tubing pump, nitrate CF manifold with dialyzer, cadmium reductor, colorimeter with flowcell, filter with a wavelength of between 520 nm and 540 nm and evaluation unit (see Figure B1).

6 Procedure

6.1 Sample preparation

Store samples at a temperature of at least - 18°C. Homogenize frozen vegetable samples in a cutter (5.1). Keep homogenized samples and vegetable juices at a temperature of at least - 18°C until just before extraction.

NOTE: Storage at -18 °C before homogenization is required to assure cell-breakage and complete extraction. A ruggedness study showed that extraction at room temperature is then sufficient.

6.2 Sample extraction

Weigh 40,0 g of frozen and homogenized sample into a 600 ml beaker. By means of a balance or gravimetric diluter (5.2), add 35,0 g of extraction buffer (4.15) to prevent conversion of nitrate into nitrite (see note), and 325,0 g of water. Homogenize (5.3) for at least 1 min, and filter sample through fluted filter paper. Analyse the extract after filtration on the same day. Proceed as described in 6.6.

NOTE: Addition of extraction buffer is only needed in case nitrite is to be determined as well. If not extraction can be performed with 360 g of water. If extraction buffer is added extracts may be kept up to 48 h at 4 °C.

6.3 Insertion of cadmium column

If necessary, before insertion, fill the column with water or buffer solution (4.16), e.g by using a syringe. Sleeve both ends with transmission tubing. Start pumping buffer solution (4.16) in the CFA-system. When all tubes are filled with reagents and all air is removed from transmission lines, connect the column to the nitrate cartridge starting on the side of the debubbler. For initial activation of the column prior to the measurement, pump the conditioner solution (4.23) through the system for about 30 min. Then pump buffer solution (4.16), colour reagent (4.18) and diluting solution (4.17) through the system.

6.4 Checking the reducing capacity of cadmium column

The CFA-system is usually ready for measurement after pumping of all reagents (4.16 to 4.18) for about 15 min. Fill the sample cups of the sampler with reducing capacity control solutions consisting of nitrate standard solutions ρ (NO_3^-) = 100 mg/l, 200 mg/l and 300 mg/l (4.20), and nitrite solutions (4.22). Use 3 sample cups for each standard level, and place them for each ion in a sequence of increasing concentration. It is recommended to insert blanks between the standard solutions and the sample test solutions.

Read the extinction for each nitrate ($A_{NO_3^-}$) and nitrite ($A_{NO_2^-}$) standard from the recorder strip chart and/or integrator. Calculate the mean extinction for each concentration level.

Calculate the reducing capacity, C_R, of the cadmium column, expressed as a percentage of reduction, by comparing the extinction values, $A_{NO_2^-}$, of the nitrite standard solutions with those of the nitrate standard solutions, $A_{NO_3^-}$, using equation (1):

$$C_R = \frac{A_{NO_2^-} \times 1{,}35 \times 100\%}{A_{NO_3^-}} \qquad (1)$$

where:

$A_{NO_3^-}$ is the response of extinction signal for nitrate;

$A_{NO_2^-}$ is the response of extinction signal for nitrite;

1,35 is the conversion factor from nitrite to nitrate (= $M_{NO_3^-}/M_{NO_2^-}$).

Regenerate the column according to the procedure described in 6.6 if the reduction capacity is below 95%. Proceed when all requirements are fulfilled. Insert a new cadmium column if the reduction capacity is below 90%.

Dispose cadmium columns at a chemical waste departement.

6.5 Regeneration of cadmium column

Immerse the tubings which carry the buffer solution (4.16) in the regeneration solution (4.25) and pump this solution through the reductor column for about 15 min. Wash out the reductor column with water followed by 0,1 mol/l HCl (4.4), and water; each washing will last about 15 min. Condition the column by pumping the conditioner solution (4.23) through the system.

6.6 Determination

6.6.1 General

The following settings are only recommended values and system layout dependent.

6.6.2 Measurement

Immerse the tubings in their respective solutions, and start pumping reagents for about 5 min. Ensure that pump tubes are filled with reagents and that all air is removed from transmission lines before switching the valve to the column. Turn on the colorimeter and adjust the signal to the blank value after 15 min. Switch on the recorder and the integrator. Fill cups of the sampler with the 7 nitrate standard solutions (4.20) and place in the sampler (5.4) followed by 1 cup with water. Include a system check for carry-over effects by placing after the water cup 2 cups with high level nitrate standard solution followed by 2 cups of low level nitrate standard solution. Subsequently fill 10 cups of the sampler with sample solutions. Place after each series of samples 1 cup filled with 400 mg/l nitrate standard solution (4.20) and 1 cup with water in order to control the drift and the baseline of the procedure, respectively.

Choose suction time and washing time to allow the signal to reach at least 95 % of the peak plateau. The wash time should be sufficiently long so that the signal between two measurements, decreases for at least 80 % of the previously measured peak height.

Prepare one reagent blank for each series.

Measure the extinction of standards and sample solutions with the spectrometer (5.4).

6.7 System shutdown

Switch off or remove the reduction column, avoiding entrapment of air bubbles. Store the reduction column filled with buffer solution (4.16). Rinse the system with water for at least 10 min. Clean the CFA-system periodically with hypochlorite solution (4.26) after the reduction column has been removed.

7 Calculation

Plot extinction responses of the standard solutions (4.20) against the corresponding concentrations. Read off nitrate-ion concentrations (C_x) of samples.

Calculate the mass fraction, w_{NO_3}, of nitrate ion in the samples, in milligrams per kilogram, with equation (2):

$$w_{NO_3} = C_x \times \frac{W + m}{m \times \rho_{20}} \qquad (2)$$

where:

C_x is the mass concentration of nitrate ions interpolated from standard plot, in milligrams per litre;

W is the mass of water and/or added extraction buffer (see 6.2), in grams;

m is the mass of test portion, in grams;

ρ_{20} is the density of the standard solution (i.e. 1,00) in grams per millilitre.

NOTE: The absorption maxima of beetroot extracts and diazotized nitrite solutions are nearly identical. Therefore nitrate or nitrite values for beetroot samples should be corrected by measuring the blank absorption value for beetroot extracts. This may be accomplished by running each sample twice with and without colour-producing agents. The extinction to be used is the difference between the two signals.

8 Precision

8.1 General

Details of the interlaboratory test of the method are summarized in annex C. The values derived from the interlaboratory test may not be applicable to other analyte concentration ranges and matrices than given in the annex.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value r in not more than 5 % of the cases.

The values are:

beetroot	$\bar{x}$ = 901 mg/kg	r = 139 mg/kg
lettuce	$\bar{x}$ = 1319 mg/kg	r = 183 mg/kg
endive	$\bar{x}$ = 1981 mg/kg	r = 94 mg/kg
beetroot	$\bar{x}$ = 2655 mg/kg	r = 246 mg/kg
lettuce	$\bar{x}$ = 2738 mg/kg	r = 252 mg/kg
lettuce	$\bar{x}$ = 4021 mg/kg	r = 271 mg/kg
spinach	$\bar{x}$ = 5197 mg/kg	r = 342 mg/kg

8.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility value R in not more than 5 % of the cases.

The values are:

beetroot	$\bar{x}$ = 901 mg/kg	R = 149 mg/kg
lettuce	$\bar{x}$ = 1319 mg/kg	R = 201mg/kg
endive	$\bar{x}$ = 1981 mg/kg	R = 209 mg/kg
beetroot	$\bar{x}$ = 2655 mg/kg	R = 338 mg/kg
lettuce	$\bar{x}$ = 2738 mg/kg	R = 252 mg/kg
lettuce	$\bar{x}$ = 4021 mg/kg	R = 420 mg/kg
spinach	$\bar{x}$ = 5197 mg/kg	R = 573 mg/kg

9 Test report

The test report shall contain at least :

a) all information necessary for the identification of the sample;
b) a reference to this European Standard or to the method used;
c) the results and the units in which the results have been expressed;
d) date and type of sampling procedure (if known);
e) date of receipt;
f) date of test;
g) if the repeatability has been verified;
h) any particular points observed in the course of the test;
i) any operations not specified in the method or regarded as optional which might have affected the results

as given in EN 12014-1.