SLOVENSKI STANDARD
SIST EN 13191-1:2001

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Živila brez maščob - Določevanje ostankov bromida - 1. del: Določevanje celotnega bromida kot anorganskega bromida

Non-fatty food - Determination of bromide residues - Part 1: Determination of total bromide as inorganic bromide

Fettarme Lebensmittel - Bestimmung von Bromidrückständen - Teil 1: Bestimmung von Gesamtbromid als anorganisches Bromid

Aliments non gras - Détermination des résidus de bromures - Partie 1: Détermination des bromures totaux en tant que bromures inorganiques

Ta slovenski standard je istoveten z: **EN 13191-1:2000**

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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Non-fatty food - Determination of bromide residues - Part 1: Determination of total bromide as inorganic bromide

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This European Standard was approved by CEN on 8 April 2000.

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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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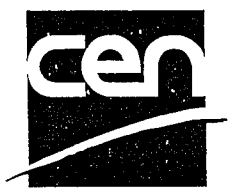

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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

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Contents

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of an identical text or by endorsement, at the latest by November 2000, and conflicting national standards shall be withdrawn at the latest by November 2000.

This European Standard "Non-fatty foods - Determination of bromide residues" consists of two parts:

Part 1: Determination of total bromide as inorganic bromide

Part 2: Determination of inorganic bromide

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.

The Annexes A and B are informative.

1 Scope

This European Standard specifies a gas chromatographic (GC) method for the determination of bromide residues (including some organic bromine present) as inorganic bromide in non-fatty foods.

Generally, the maximum residue levels are expressed in terms of bromide ion from all sources but not including covalently bound bromine.

The method is applicable to beets, carrots, chicory, endives, cereal grains, lettuce, potatoes, spinach, strawberries and tomato. It has been validated in an interlaboratory test on lettuce [1].

2 Normative reference

This draft 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 draft European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

3 Principle

An aqueous ethanolic extract of the test portion is evaporated to dryness and the residue is ashed in the presence of sodium hydroxide. The ash is solubilized with sulfuric acid and the solution is treated with ethylene oxide in di-isopropyl ether. Inorganic bromide is converted to 2-bromoethanol, which is analyzed by gas chromatography with electron-capture detection [2].

4 Reagents

4.1 General and safety aspects

Unless otherwise specified, use reagents of recognized analytical grade, preferably for pesticide residue analysis, and water of grade 2 according to EN ISO 3696.

Take every precaution to avoid possible contamination of water, solvents, inorganic salts, etc. by plastics and rubber materials. Use only glass containers for storage and handling of all water and reagents.

4.2 Ethanol, $\varphi(C_2H_5OH) = 96\ \%\ (V/V)$

4.3 Sodium hydroxide solution, $c(NaOH) = 0{,}2$ mol/l

4.4 Sulfuric acid, $c(H_2SO_4) = 0{,}6$ mol/l

4.5 Acetone

4.6 Di-isopropyl ether, peroxide-free.

Before use, check each newly opened bottle by injecting the same volume, e.g. 5 µl, into the gas chromatograph as used in 6.3. If interfering peaks are observed, distill over potassium hydroxide.

WARNING: Di-isopropyl ether is extremely flammable. Store protected from light. Unstable peroxides can form upon longer standing or exposure to sunlight in bottles and can result in explosion risk. Work always in a well-ventilated fume hood.

4.7 Ethylene oxide, $\varphi(C_2H_4O)$ of at least 99,5 % volume fraction, in pressurized can fitted with valve. Store at approximately - 20 °C.

WARNING: Ethylene oxide is a highly reactive and cancerogenic gas. Work always in a well-ventilated fume hood. Consult the safety data sheets of the manufacturer for information.

To destroy excess ethylene oxide solution, add a surplus of sodium chloride solution, shake several times and allow to stand for some hours.

4.8 Ethylene oxide solution

In a well-ventilated fume hood, pour 96 ml of ice-cold di-isopropyl ether (4.6) into a 100 ml volumetric flask, and add ethylene oxide (4.7) dropwise to the mark from the completely inverted, ice-cold pressurized can and mix well. Store at approximately 4 °C. Prepare fresh daily.

4.9 Ammonium sulfate

4.10 Sodium sulfate, anhydrous. Heat for 5 h at 500 °C.

4.11 2-Bromoethanol standard solution, in acetone, ρ (C_2H_5BrO) = 1 mg/l.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.1 Chopper

5.2 Homogenizer or high speed blender, fitted with glass jar.

5.3 Centrifuge, provided with glass tubes of capacity 100 ml, and capable of producing a centrifugal acceleration of at least 7 000 *g* at the basis of the centrifuge tubes.

5.4 Nickel crucibles, e.g. 5,5 cm high, inner diameter 6 cm.

5.5 Water bath, capable of being maintained at 100°C.

5.6 Laboratory oven, capable of being maintained at approximately 80 °C, with ventilation.

5.7 Muffle furnace, capable of being maintained at approximately 500 °C.

5.8 Conical flask, 250 ml, with ground joint.

5.9 Test tubes, 10 ml and 25 ml, with ground joint.

5.10 Gas chromatograph, equipped with an electron-capture detector.

6 Procedure

6.1 Ashing

Chop the test sample using the chopper (5.1), avoiding loss of juice. Transfer a test portion of 50 g into the blender cup (5.2) and add 50 ml of water and 50 ml of ethanol (4.2). Blend for 2 min to 3 min and centrifuge at 7 000 *g*. Calculate the total volume of supernatant taking into account the water content of the sample. Transfer an aliquot of supernatant corresponding to 15 g of the test portion, into a nickel crucible (5.4) and add 5 ml of sodium hydroxide solution (4.3). Place the nickel crucible (without lid) on a boiling water bath (5.5) until the liquid is evaporated. Place the nickle crucible first overnight in a well-ventilated oven (5.6) at approximately 80 °C, and then in a muffle furnace (5.7) for 15 min at 500 °C.

> **WARNING:** Good ventilation in the oven is necessary, as otherwise explosions could occur due to the formation of flammable mixtures of ethanol and air.

During the ashing procedure, air shall be allowed to come in contact with the evaporated residue, as otherwise mineralization will be incomplete. The colour of the ash should be grey to dark-grey.

> NOTE 1: Sometimes, e.g. in the case of beet samples, the evaporated residue has been found to ignite in the furnace due to inadequate heat transfer. Ignition leads to appreciable losses of bromide. In this case, a smaller test portion should be used.
>
> NOTE 2: Reduction of the supernatant corresponding to approximately 1 g of the test portion is necessary when samples with high content of organic matter are analysed, because otherwise ashing will be incomplete.

6.2 Derivatization

Allow the ash to cool and add 5 ml of sulfuric acid (4.4). Mix the ash well with the liquid and transfer the content of the crucible to a conical flask (5.8), using 20 ml of acetone (4.5) for rinsing. Add 5 ml of ethylene oxide solution (4.8). Stopper the flask, mix the contents by shaking and allow to stand for 1 h at room temperature.

Transfer approximately 10 ml of the upper organic phase to a 25 ml test tube (5.9) and add 2 g of ammonium sulfate (4.9). Stopper the tube and shake vigorously for 2 min. Allow to settle and transfer approximately 5 ml of the supernatant to a 10 ml test tube. Add 1 g of sodium sulfate (4.10), stopper the tube, shake well and allow to stand for 30 min at room temperature (sample test solution).

6.3 Gas chromatography

Inject equal volumes *(B)* of the sample test solution derived from 6.2 and of dilutions of 2-bromoethanol standard solution (4.11) into the gas chromatograph.

Make sure that the gas chromatographic conditions (column length, stationary phase type, injector, detector and column temperatures, gas flow rates, etc.) are such that the separation of 2-bromoethanol from possible interfering peaks originating from the samples is as complete as possible.

Typical gas chromatographic conditions are given in Annex A.

NOTE 1: Due to their high separation efficiency, capillary columns are preferably used.

NOTE 2: 2-chloroethanol is formed from chloride ions naturally occurring in the sample material. Its peak, however, has a shorter retention time and does not interfere with the determination.

6.4 Test for interferences and recoveries

Prepare reagent blanks and carry out spiked recovery tests at levels appropriate to the maximum residue level.

The chromatogram of the reagent blank should not show a peak at the retention time of 2-bromoethanol.

NOTE: Occasionally, a tailing peak with a longer retention time appears which is due to ethylene glycol and does not interfere with the determination.

7 Evaluation of results

Measure the peak height (or peak area) obtained from the sample test solution and compare it with the peak heights (or peak areas) obtained for appropriate dilutions of the 2-bromoethanol standard solution (4.11). Based on this quotient, calculate the mass *(A)* of 2-bromoethanol, in nanograms, present in the injected sample test solution.

Calculate the mass fraction w of bromide, in milligrams per kilogram of sample, using equation (1):

$$w = \frac{A \times 25 \times 0,639}{B \times 15} \quad (1)$$

where:

- A is the mass of 2-bromoethanol in the injected volume of the sample test solution, in nanograms;
- B is the injection volume, in microlitres ;
- 0,639 is the factor for conversion of 2-bromoethanol to bromide ;
- 25 is the volume of the organic solvents added in 6.2, in millilitres (20 ml of acetone and 5 ml of ethylene oxide solution).
- 15 is the mass of the aliquot of the test portion ashed in the nickel crucible, in grams ;

If the results indicate that the amount of residue approaches or exceeds the maximum residue level, examine at least two further test portions.

8 Confirmatory tests

The bromide content can be confirmed with the derivatization method described in EN 13191-2:2000 [3] using propylenoxide as reagent.

9 Precision

9.1 General

Details of the interlaboratory test of the precision of the method according to ISO 5725:1986 [4] are summarized in annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in annex B.

9.2 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 limit r in not more than 5 % of the cases.

The values are:	lettuce from untreated soil	$\bar{x}$ =	13,3 mg/kg	r =	3,6 mg/kg
	lettuce from treated soil	$\bar{x}$ =	209 mg/kg	r =	35,0 mg/kg

9.3 Reproducibility

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

The values are:	lettuce from untreated soil	$\bar{x}$ =	13,3 mg/kg	R =	6,9 mg/kg
	lettuce from treated soil	$\bar{x}$ =	209 mg/kg	R =	75,0 mg/kg

10 Test report

The test report shall contain at least the following information:

- all information necessary for the identification of the sample;
- a reference to this European Standard or to the method used;
- the results and the units in which the results have been expressed;
- date and type of sampling procedure (if known);
- date of receipt of sample in the laboratory;
- date of test
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results.

Annex A (informative)
Examples for appropriate gas chromatographic operating conditions

A.1 The following gas chromatographic operating conditions have been proven to be satisfactory.

A.2 Packed glass columns

Column size	1,80 m x 2 mm
Column filling	15 % PPG (UCON LB 550-X)®[1)] or 10 % Carbowax 20-M on Chromosorb W-HP, 150 µm to 190 µm (80 mesh to 100 mesh)
Column temperature	120 °C
Injector temperature	130 °C
Carrier gas	nitrogen or argon/methane, flow rate 60 ml/min

Column size	1,80 m x 4 mm
Column filling	Tenax G/c, 190 µm to 250 µm (60 mesh to 80 mesh)
Column temperature	165 °C
Injector temperature	175 °C
Carrier gas	nitrogen, flow rate 65 ml/min

Column size	1,80 m x 2 mm
Column filling	10 % OV-330 on Chromosorb W-HP, 150 µm to 190 µm (80 mesh to 100 mesh)
Column temperature	110 °C
Injector temperature	150 °C
Carrier gas	nitrogen or argon/methane, flow rate 30 ml/min

Column size	1,50 m x 3 mm
Column filling	10 % Carbowax 20-M on Chromosorb W-HP, 120 µm to 150 µm (100 mesh to 120 mesh)
Column temperature	130 °C
Injector temperature	200 °C
Carrier gas	nitrogen, flow rate 40 ml/min

A.3 Capillary columns (fused silica)

Column size	60 m x 0,53 mm
Column stationary phase	Restek Rt_x-1701®[1)], film thickness 3,0 µm
Column temperature	programmed to rise from 35 °C to 225 °C with 5 °C/min
Injector temperature	250 °C
Carrier gas	hydrogen, flow rate 5 ml/min

Column size	30 m x 0,53 mm
Column stationary phase	Restek Stabilwax, film thickness 1,0 µm
Column temperature	programmed to rise from 40 °C to 225 °C with 5 °C/min
Injector temperature	250 °C
Carrier gas	hydrogen, flow rate 5 ml/min

[1)] UCON LB 550-X® and Restek RTx-1701® are examples of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.