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International Standard



6635

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**Fruits, vegetables and derived products — Determination of nitrite and nitrate content — Molecular absorption spectrometric method**

*Fruits, légumes et produits dérivés — Détermination de la teneur en nitrite et en nitrate — Méthode spectrométrique d'absorption moléculaire*

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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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6635 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

# Fruits, vegetables and derived products — Determination of nitrite and nitrate content — Molecular absorption spectrometric method

## 1 Scope and field of application

This International Standard specifies a method for the determination of the nitrite and nitrate content of fruits, vegetables and derived products.

## 2 Principle

### 2.1 Determination of nitrite

Extraction of a test portion with hot water, precipitation of the proteins by addition of solutions of potassium hexacyanoferrate(II)<sup>1)</sup> and zinc acetate, and filtration of the precipitate. Addition of sulfanilamide chloride and *N*-(1-naphthyl)ethylenediamine dihydrochloride to the filtrate, and spectrometric measurement of the red complex obtained in the presence of nitrite at a wavelength of 538 nm.

### 2.2 Determination of nitrate

Extraction of a test portion with hot water, precipitation of the proteins by addition of solutions of potassium hexacyanoferrate(II)<sup>1)</sup> and zinc acetate, and filtration of the precipitate. Reduction of the nitrate to nitrite by metallic cadmium. Addition of sulfanilamide chloride and *N*-(1-naphthyl)ethylenediamine dihydrochloride to the filtrate, and spectrometric measurement of the red complex obtained in the presence of nitrite at a wavelength of 538 nm.

## 3 Reagents

All reagents shall be of recognized analytical grade and the water used shall be distilled water or water of at least equivalent purity.

### 3.1 Disodium tetraborate, saturated solution.

Dissolve 50 g of disodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 1 000 ml of tepid water and cool to room temperature.

### 3.2 Potassium hexacyanoferrate(II), solution.

Dissolve 106 g of potassium hexacyanoferrate(II) trihydrate [ $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ ] in water in a 1 000 ml one-mark volumetric flask and make up to the mark with water.

### 3.3 Zinc acetate, solution.

Dissolve 220 g of zinc acetate dihydrate [ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ] in a mixture of water and 30 ml of glacial acetic acid in a 1 000 ml one-mark volumetric flask and make up to the mark with water.

### 3.4 Reagent for colour development.

NOTE — Solutions I and II can be stored in well closed, dark brown bottles for up to 1 week.

#### 3.4.1 Solution I.

Dissolve 0,4 g of sulfanilamide in about 160 ml of water in a 200 ml one-mark volumetric flask by heating on a boiling water-bath. Cool and filter if necessary, then add, while agitating, 20 ml of hydrochloric acid ( $\rho_{20} = 1,19$  g/ml). Dilute to the mark with water and mix.

#### 3.4.2 Solution II.

Dissolve 0,1 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride ( $\text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$ ) in water in a 100 ml one-mark volumetric flask. Make up to the mark with water and mix.

#### 3.4.3 Solution III.

Dilute 445 ml of hydrochloric acid ( $\rho_{20} = 1,19$  g/ml) to the mark with water in a 1 000 ml one-mark volumetric flask.

### 3.5 Zinc, in rods about 150 mm long and of diameter 5 to 7 mm.

### 3.6 Cadmium sulfate, solution.

Dissolve 40 g of cadmium sulfate octahydrate ( $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) in water in a 200 ml one-mark volumetric flask and make up to the mark with water.

1) Formerly known as potassium ferrocyanide.

### 3.7 Cadmium.

Place the zinc rods (3.5) in a 250 ml tall-form beaker containing 200 ml of the cadmium sulfate solution (3.6) and leave for 1 h, regularly scraping off the reduced cadmium, using the spatula (4.1.7).

Pulverize in the beaker using the stirring rod (4.1.8). Before use, treat the cadmium collected from the zinc rods with hydrochloric acid,  $c(\text{HCl}) = 0,1 \text{ mol/l}^{1)}$ , then wash several times with water.

Pulverize the cadmium collected and keep it in water.

### 3.8 Ammoniacal buffer solution, pH 9,6.

Dissolve 37,4 g of ammonium chloride ( $\text{NH}_4\text{Cl}$ ) in about 900 ml of water in a 1 000 ml one-mark volumetric flask. Adjust the solution to pH 9,6 with concentrated ammonia solution,  $\rho_{20} = 0,88 \text{ g/ml}$ , and make up to the mark with water.

### 3.9 Sodium nitrite, standard solution.

Weigh, to the nearest 1 mg, 3 g of sodium nitrite ( $\text{NaNO}_2$ )<sup>2)</sup>, previously dried at  $115 \pm 5 \text{ }^\circ\text{C}$  to constant mass, and dissolve it in 50 ml of water. Transfer quantitatively to a 1 000 ml one-mark volumetric flask, shake well and make up to the mark.

Pipette 5 ml of this solution into a 1 000 ml one-mark volumetric flask and make up to the mark.

1 ml of this standard solution contains 10  $\mu\text{g}$  of nitrite ion ( $\text{NO}_2^-$ ).

This solution is unstable and shall be prepared at the time of use.

## 4 Determination of nitrite content

### 4.1 Apparatus

All glassware shall be thoroughly washed and rinsed in distilled or deionized water to ensure that it is free of nitrites and nitrates.

Usual laboratory equipment, and in particular

**4.1.1 Volumetric flasks**, of capacities 50 and 200 ml, complying with the requirements of ISO 1042.

**4.1.2 Beakers**, of capacity 200 ml.

**4.1.3 Volumetric pipettes**, of capacities 1; 2; 3; 5; and 10 ml, complying with the requirements of ISO 648.

**4.1.4 Fluted filter paper**, nitrite-free.

**4.1.5 Conical flasks**, of capacity 25 ml, fitted with ground-glass stoppers.

**4.1.6 Boiling water-bath**.

**4.1.7 Spatula**, the end of which is coated with PTFE.

**4.1.8 Stirring rod**, with the end flattened and covered with PTFE.

**4.1.9 Spectrometer**, with selectors for continuous or discontinuous variation, suitable for measurements at a wavelength of 538 nm, fitted with cells of thickness 1 cm.

**4.1.10 Analytical balance**.

### 4.2 Procedure

#### 4.2.1 Preparation of the test sample

Mix the laboratory sample well. If necessary, first remove stones and hard seed-cavity walls and pass the laboratory sample through a mechanical grinding mill.

Allow frozen or deep frozen products to thaw in a closed vessel and add the liquid formed during this process to the product before homogenization.

#### 4.2.2 Test portion

Take, by means of a pipette (4.1.3), 1 to 10 ml, or weigh 1 to 10 g, of the test sample (4.2.1), according to the expected nitrite content.

#### 4.2.3 Preparation of filtrate

Transfer the test portion (4.2.2) quantitatively to one of the beakers (4.1.2) and add 5 ml of the disodium tetraborate solution (3.1) and about 100 ml of hot (70 to 80  $^\circ\text{C}$ ) water.

Heat the beaker for 15 min on the boiling water-bath (4.1.6), shaking from time to time. Add, successively, 2 ml of the potassium hexacyanoferrate(II)<sup>3)</sup> solution (3.2) and 2 ml of the zinc acetate solution (3.3), shaking after each addition.

Cool. Transfer quantitatively to a 200 ml one-mark volumetric flask (4.1.1), rinse and make up to the mark with the rinsing water. Shake.

Filter through the fluted filter paper (4.1.4) and, if necessary, repeat the filtration until a clear liquid is obtained.

1) Hitherto expressed as "0,1 N".

2) It may be necessary to verify the purity of this reagent.

3) Formerly known as potassium ferrocyanide.

#### 4.2.4 Determination

Transfer, by means of a pipette (4.1.3), an aliquot portion of the filtrate (10 ml at least) to a 50 ml one-mark volumetric flask (4.1.1). Dilute to approximately 30 ml with water.

Add, by means of a pipette (4.1.3), 5 ml of solution I (3.4.1), then 3 ml of solution III (3.4.3) and mix.

Leave the solution at ambient temperature protected from light.

Add 1 ml of solution II (3.4.2). Mix carefully and leave the solution for 3 min at ambient temperature, protected from light. Make up to the mark with water and mix.

Within 15 min, measure the absorbance of the solution using the spectrometer (4.1.9).

Subtract the value for the blank test (see 4.2.5) and read the mass of nitrite from the calibration graph (see 4.2.6).

#### 4.2.5 Blank test

Carry out a blank test, proceeding as specified in 4.2.3 and 4.2.4, but replacing the test portion by 10 ml of water.

#### 4.2.6 Preparation of the calibration graph

Into a series of six 50 ml one-mark volumetric flasks (4.1.1), place 0; 0,5; 1; 2; 2,5; and 3 ml of the standard sodium nitrite solution (3.9) and 30; 29,5; 29; 28; 27,5; and 27 ml of water respectively. Proceed as specified in 4.2.4, from the second paragraph onwards.

Plot a graph, having the masses, in micrograms, of nitrite in the calibration solutions as abscissae, and the corresponding values of absorbance, measured by the spectrometer (4.1.9), as ordinates.

### 4.3 Expression of results

#### 4.3.1 Method of calculation and formula

The nitrite content, expressed as milligrams of nitrite ion ( $\text{NO}_2^-$ ) per kilogram, or in milligrams per litre, of product, is given by the formula

$$m_1 \times \frac{200}{V_1 \times m_0}$$

or

$$m_1 \times \frac{200}{V_1 \times V_0}$$

where

$m_0$  is the mass, in grams, of the test portion (4.2.2);

$m_1$  is the mass, in micrograms, of nitrite ion ( $\text{NO}_2^-$ ) contained in the aliquot portion ( $V_1$ ) of filtrate taken (see 4.2.4), read from the calibration graph (see 4.2.6);

$V_0$  is the volume, in millilitres, of the test portion (4.2.2);

$V_1$  is the volume, in millilitres, of the aliquot portion of filtrate taken (4.2.4).

#### 4.3.2 Reproducibility

The difference between the results obtained in two different laboratories on the same sample shall not differ by more than 3 % (relative).

## 5 Determination of nitrate content

### 5.1 Apparatus

The apparatus specified in 4.1, and

#### 5.1.1 Vertical mechanical agitator.

#### 5.1.2 Filter paper, for slow filtration, nitrite- and nitrate-free.

### 5.2 Procedure

#### 5.2.1 Preparation of test sample, test portion and preparation of filtrate

Proceed as described in 4.2.1, 4.2.2 and 4.2.3.

#### 5.2.2 Determination

Transfer, by means of a pipette (4.1.3), an aliquot portion of the filtrate (10 ml or less)<sup>1)</sup> to a 25 ml conical flask (4.1.5) into which about 2 g of the cadmium (3.7) and 5 ml of the buffer solution (3.8) have been previously placed. Stopper the flask and shake on the vertical mechanical agitator (5.1.1) for 5 min.

Filter through the filter paper (5.1.2) and collect the filtrate in a 50 ml one-mark volumetric flask (4.1.1). Rinse the filter paper several times with small quantities of water and collect the rinsing water in the flask. Make up to the mark.

Proceed with the determination of total nitrite as described in the first five paragraphs of 4.2.4, using 10 ml of the test solution.

Subtract the value for the blank test (see 5.2.3) and read the total mass of nitrite from the calibration graph (see 4.2.6).

#### 5.2.3 Blank test

Carry out a blank test, proceeding as specified in 5.2.1 and 5.2.2, but replacing the test portion by 10 ml of water.

1) This aliquot portion shall contain between 30 and 120  $\mu\text{g}$  of nitrate ion ( $\text{NO}_3^-$ ).

### 5.3 Expression of results

#### 5.3.1 Method of calculation and formula

The nitrate content, expressed as milligrams of nitrate ion ( $\text{NO}_3^-$ ) per kilogram, or in milligrams per litre, of product, is given by the formula

$$1,348 \left( \frac{m_2 \times 10\,000}{V_3 \times V_2 \times m_0} - \frac{m_1 \times 200}{V_1 \times m_0} \right)$$

or

$$1,348 \left( \frac{m_2 \times 10\,000}{V_3 \times V_2 \times V_0} - \frac{m_1 \times 200}{V_1 \times V_0} \right)$$

where

$m_2$  is the total mass of nitrite, in micrograms of nitrite ion ( $\text{NO}_2^-$ ), contained in the volume ( $V_2$ ) of test solution taken (see 5.2.2), read from the calibration graph (see 4.2.6);

$V_2$  is the volume, in millilitres, of the test solution (see 5.2.2) taken for the spectrometric measurement;

$V_3$  is the volume, in millilitres, of the aliquot portion of the filtrate taken for the preparation of the test solution (see 5.2.2);

$m_0$ ,  $m_1$ ,  $V_0$  and  $V_1$  have the same meanings as in 4.3.1;

1,348 is the ratio between the relative molecular masses of nitrate ion ( $\text{NO}_3^-$ ) and nitrite ion ( $\text{NO}_2^-$ ).

#### 5.3.2 Reproducibility

See 4.3.2.

## 6 Test report

The test report shall show the method used and the results obtained, indicating clearly the method of expression used. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.