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Fruits, vegetables and derived products — Determination of arsenic content — Method using hydride generation atomic absorption spectrometry

Fruits, légumes et produit dérivés — Détermination de la teneur en arsenic — Méthode par spectrométrie d'absorption atomique à génération de hydrure

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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International Standard ISO 17239 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 3, *Fruit and vegetable products*.

Fruits, vegetables and derived products — Determination of arsenic content — Method using hydride generation atomic absorption spectrometry

1 Scope

This International Standard specifies a hydride generation atomic absorption spectrometric method for the determination of the arsenic content of fruits, vegetables and derived products.

NOTE The method of arsenic determination in fruit, vegetables and derived products is based on AOAC Official Methods for Analysis (see Reference [1]).

2 Principle

Organic matter is decomposed by digestion with HNO_3 in a closed system. Arsenic(V) is reduced to arsenic(III) with potassium iodide, and hydrides of arsenic are generated by the action of sodium borohydride prior to atomization in a flame-heated quartz cell. Measurement is by atomic absorption spectrometry.

3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

3.1 Nitric acid, concentrated, (ρ_{20} = 1,38 g/ml).

3.2 Hydrochloric acid, 8 mol/l solution.

Place 66 ml of concentrated hydrochloric acid (ρ_{20} = 1,19 g/ml) in a 100 ml one-mark volumetric flask and make up to the mark with water. Mix.

3.3 Hydrochloric acid, diluted 1 + 1, by volume.

Mix one volume of concentrated hydrochloric acid (ρ_{20} = 1,19 g/ml) with one volume of water.

3.4 Magnesium chloride, 37,5 mg/ml solution.

Dissolve, in a 100 ml one-mark volumetric flask, 3,75 g of magnesium oxide by gradually adding approximately 20 ml of hydrochloric acid solution (3.2) and make up to the mark with water. Mix.

3.5 Magnesium nitrate, 75 mg/ml solution.

Dissolve, in a 50 ml one-mark volumetric flask, 3,75 g of magnesium oxide with approximately 30 ml of water. Slowly add approximately 10 ml of nitric acid (3.1) and mix. Make up to the mark with water.

3.6 Sodium borohydride, 4 % solution.

Dissolve, in a 100 ml one-mark volumetric flask, 4 g of $NaBH_4$ in 4 % sodium hydroxide. Mix and make up to the mark with 4 % sodium hydroxide.

3.7 Potassium iodide, 20 % solution.

Dissolve, in a 100 ml one-mark volumetric flask, 20 g of potassium iodide in water. Mix and make up to the mark with water. Prepare just before use.

3.8 Arsenic, standard solution corresponding to 1,0 mg of arsenic per millilitre.

4 Apparatus

Rinse all glassware before using with a nitric acid solution (diluted 1 + 1), followed by a thorough rinse with water.

Decontaminate the digestion vessels by digesting with reagents to be used in digestion. Rinse thoroughly with water.

Usual laboratory apparatus and, in particular, the following.

4.1 Mechanical grinder, the inside and blades of which are coated with polytetrafluoroethylene (PTFE).

4.2 Atomic absorption spectrometer, provided with an air/acetylene burner (10 cm) and hydrogen-nitrogen-entrained air flames, suitable for measurement at a wavelength of 193,7 nm and equipped with a deuterium-arc background correction.

4.3 Decomposition vessel, 70 ml (see Figure A.1), consisting of a stainless steel body supporting a polytetrafluoroethylene (PTFE) crucible having a screw-cap with a PTFE liner to provide a PTFE sealing surface.

A PTFE spout is snapped on outside the rim to permit a quantitative transfer of the contents without contacting the metal parts.

4.4 Oven, capable of maintaining a temperature of 150 °C.

4.5 Furnace, capable of maintaining a temperature of 450 °C.

4.6 Hydride generator, (see Figure A.2), constructed from the following:

4.6.1 Flat-bottom flask, borosilicate glass, 50 ml capacity.

4.6.2 Stoppers, two-hole (one through the centre), No 9 rubber stopper, with a gas-outlet tube of polyethylene tubing, 100 mm long with a 3,2 mm id, fitted through the centre hole.

4.6.3 Reagent-cup assembly, consisting of the bottom 25 mm of a polyethylene test tube with a hole in the bottom, through which the lower end of a gas-outlet tube is placed so that 3 mm of a tube protrudes below the lower edge of the test tube.

Connect the other end of the outlet tubing to the atomic absorption spectrometer (4.2) with 500 mm Tygon[1)] tubing by cutting the auxiliary line approximately 75 mm from the mixing chamber and attaching the tubing.

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1) Tygon is the trade name of a product. This information is only given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4.6.4 **N_2 inlet tube**, made by sealing the bottom end of a segment of polyethylene tubing, 75 mm long with a 3,2 mm id, with a burner and then punching several holes in the sealed end with a 21-gauge needle which is inserted through the second hole of the stopper. Alternatively, prepare a segment of polyethylene tubing 500 mm long with a 1,6 mm id in a similar manner which is secured in place in the stopper with a hole-through septum.

4.6.5 **Generator mount** (optional), consisting of a segment of pipe, 64 mm long with a 1,3 mm id, secured to a laboratory ring stand by means of a clamp holder. Insert an extension clamp into the pipe and attach another clamp to the back of clamp to hold clamp in place and to serve as a handle. The clamp is now free to rotate 180°.

Attach the rubber stopper of the hydride generator to the extension clamp with a stiff wire and position just at the level of the clamp jaws. In operation, place the flask of the generator between the jaws of the extension clamp, insert the stopper firmly into the neck of the flask, then tighten the clamp jaws around neck of the flask. The unit can be rapidly and uniformly inverted by rotating the handle on extension clamp, thus allowing the sample and the sodium borohydride to mix rapidly and reproducibly.

4.7 **Pipettes**, of appropriate capacities.

4.8 **One-mark volumetric flasks**, 10 ml, 50 ml and 200 ml capacity.

4.9 **Analytical balance**.

5 Sample

It is important that the laboratory receive a sample that is truly representative and that has not been damaged or changed during transport or storage.

6 Procedure

6.1 Preparation of test sample

Thoroughly mix the laboratory sample. If necessary, first remove any seeds, stalks and hard seed-cavity walls, and then grind in the mechanical grinder (4.1).

Frozen or deep-frozen products shall be previously thawed in a closed vessel, and the liquid formed during this process shall be added to the product before mixing.

6.2 Test portion

Weigh, to the nearest 0,01 g, 0,3 g (dry basis) of the test sample (6.1) into the decomposition vessel (4.3).

6.3 Decomposition

CAUTION — Do not exceed manufacturers' specification of 0,3 g of solids in a 70 ml vessel. Proceed cautiously with new or untried applications. Let such sample stand with nitric acid (3.1) overnight or heat on a hot plate cautiously until any vigorous reaction subsides. Then proceed with the closed vessel digestion. Open the vessel in a hood since nitrogen oxides are released.

If the test portion contains ethanol, remove the ethanol by evaporation. Add 5 ml of nitric acid (3.1), close the vessel with a lid, and heat at 150 °C in the oven (4.4) for 2 h.

Cool in a hood, remove the vessel from the jacket, and transfer the contents to a 10 ml volumetric flask (4.8).

Add 4 ml of water to the vessel, cover with the lid, and while holding the lid tightly against the rim, invert several times, and add rinse to the flask.

Dilute to the volume mark with water and mix.

6.4 Blank test

Prepare a blank solution using the same decomposition procedure (6.3), but replacing the test portion (6.2) with 1 ml of water.

6.5 Determination

6.5.1 Preparation of calibration graph

Dilute the standard arsenic solution (3.8) with water to obtain five solutions with an arsenic concentration of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, or 5 µg/ml.

Add 2,0 ml of magnesium chloride solution (3.4) to each of a series of six 50 ml one-mark volumetric flasks (4.8). Add 50 µl of one of the standard solutions with an arsenic concentration of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, or 5 µg/ml to flasks two to six.

These six flasks now contain 0 µg, 0,05 µg, 0,10 µg, 0,15 µg, 0,20 µg, or 0,25 µg of arsenic. Other amounts of arsenic may be used depending on sensitivity of a system.

Add 0,1 ml of potassium iodide solution (3.7) to each flask, mix, and let stand for at least 2 min.

Connect the generator (4.6) to the instrument (4.2) and adjust pressures and flows as in Table 1. Operate the instrument according to the manufacturer's instructions, with a lamp in place and the recorder set for 20 mm/min.

Add 2,0 ml of sodium borohydride solution (3.6) to the reagent dispenser of the generator, and insert the rubber stopper tightly into the neck of the flask containing standard.

With single, rapid, smooth motion, invert the flask, mixing the solution with the standard. (This operation must be performed reproducibly). A sharp, narrow absorbance peak will appear immediately.

When recorder pen returns to the baseline, remove stopper from the flask and rinse the reagent dispenser with water from squeeze bottle; then suck out water.

Proceed with the next standard. When a series is complete, rinse the glassware thoroughly.

Plot a calibration curve of the arsenic concentration, expressed in micrograms, against absorbance.

Table 1 — Flow rates and pressures for arsenic determination

Gas	Pressure kPa		Flow rate l/min
	Tank, ψ_T	AA control box, ψ_C	
H_2	140	70	4
N_2	280	210	10

6.5.2 Spectrometric measurement

Pipette an aliquot of the digested sample (6.3) or blank test (6.4) into the flask (4.6.1) and add 1 ml of magnesium nitrate solution (3.5). Heat on a hot plate at low heat to dryness; then increase heat to the maximum temperature (about 375 °C). Place the flask in a furnace (4.5) set for 450 °C to oxidize any carbonaceous matter and to decompose the excess magnesium nitrate for $\geqslant$ 30 min. Cool, dissolve the residue in 2,0 ml of hydrochloric acid solution (3.2). Then add 0,1 ml of potassium iodide solution (3.7) and let stand for at least 2 min.

Connect the generator (4.6) to the instrument (4.2), adjust the pressures and flows as in Table 1 and proceed with the calibration graph preparation (6.5.1).

Read the arsenic content of the test sample or blank from the calibration graph (6.5.1).

7 Calculation

The arsenic content of the sample, w, expressed in milligrams per kilogram of the product, is given by the equation:

$$w = \frac{m_1 - m_2}{m_0}$$

where

m_1 is the mass, expressed in micrograms, of arsenic in the test portion read from the calibration graph;

m_2 is the mass, expressed in micrograms, of arsenic in the blank solution read from the calibration graph;

m_0 is the mass, expressed in grams, of the test portion.

8 Precision

8.1 Interlaboratory test

Details of interlaboratory tests on the method are summarized in Annex B. The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given in Annex B.

8.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will, in not more than in 5 % of the cases, exceed the value of the repeatability limit r given in Table B.1.

8.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in the different laboratories by different operators using different equipment will, in not more than in 5 % of the cases, exceed the value of the reproducibility limit R given in Table B.1.