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Fruits, vegetables and derived products — Determination of copper content — Method using flame atomic absorption spectrometry

Fruits, légumes et produits dérivés — Détermination de la teneur en cuivre — Méthode par spectrométrie d'absorption atomique avec flamme

Reference number
ISO 7952:1994(E)

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.

International Standard ISO 7952 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 3, *Fruit and vegetable products*.

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Fruits, vegetables and derived products — Determination of copper content — Method using flame atomic absorption spectrometry

1 Scope

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

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5515:1979, *Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Wet method.*

3 Principle

Decomposition of organic matter by either a dry or a wet method and determination of the Cu(II) cation content by flame atomic absorption spectrometry.

4 Reagents

All reagents shall be of recognized analytical grade and, in particular, shall be free from copper. The water used shall have been distilled twice in borosilicate glass apparatus, or shall be water of at least equivalent purity.

4.1 Sulfuric acid, concentrated ($\rho_{20} = 1{,}84$ g/ml).

4.2 Nitric acid, concentrated ($\rho_{20} = 1{,}38$ g/ml).

4.3 Hydrochloric acid, diluted 1 + 1 (V/V).

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

4.4 Hydrochloric acid, approximately 0,1 mol/l solution.

Place 17 ml of dilute hydrochloric acid (4.3) into a 100 ml one-mark volumetric flask and make up to the mark with water. Mix.

4.5 Copper, standard solution corresponding to 1 g of copper per litre.

Dissolve in a 1 000 ml one-mark volumetric flask 3,929 g of copper sulfate pentahydrate ($CuSO_4.5H_2O$) in doubly distilled water. Make up to the mark with water and mix.

Store this solution in a borosilicate glass bottle fitted with a ground-glass stopper.

1 ml of this standard solution contains 1 mg of Cu.

It is also possible to prepare a standard solution in the following ways.

a) Dissolve in a 1 000 ml one-mark volumetric flask 1,000 g of metallic copper in 50 ml of a 5 mol/l solution of nitric acid. Make up to the mark with doubly distilled water and mix. Store this solution in a polyethylene bottle.

b) Dissolve in a 1 000 ml one-mark volumetric flask 3,798 g of copper nitrate trihydrate [$Cu(NO_3)_2.3H_2O$] in 250 ml of doubly distilled water. Make up to the mark with water and mix. Store this solution in a polyethylene bottle.

5 Apparatus

Before use, wash the dishes and all glassware with warm (70 °C to 80 °C) concentrated nitric acid (4.2) and rinse with doubly distilled water.

Usual laboratory apparatus and, in particular, the following.

5.1 Mechanical grinder, the inside and blades of which are coated with polytetrafluoroethylene.

5.2 Round-bottom flasks, of 250 ml, 500 ml or 1 000 ml capacity.

5.3 Dishes, made of platinum or quartz, of 70 mm diameter.

5.4 One-mark volumetric flasks, of 50 ml capacity.

5.5 Pipettes, of appropriate capacity.

5.6 Centrifuge tubes, of 30 ml capacity, provided with acid-resistant stoppers.

5.7 Water bath, capable of being maintained at a temperature from 20 °C up to boiling point.

5.8 Electrical muffle furnace, capable of being maintained at a temperature of 525 °C ± 25 °C, and preferably capable of being temperature programmed in progressive steps from 20 °C to 525 °C ± 25 °C.

5.9 Laboratory centrifuge, capable of maintaining a rotational frequency of 1 600 min^{-1}, and suitable for use with the centrifuge tubes (5.6).

5.10 Atomic absorption spectrometer, provided with an air/acetylene burner, suitable for measurements at a wavelength of 324,7 nm.

5.11 Analytical balance, capable of weighing to an accuracy of ± 0,01 g.

6 Preparation of test sample

Mix well the laboratory sample. If necessary, first remove any seeds and hard seed-cavity walls, and then grind in the mechanical grinder (5.1).

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.

7 Procedure

7.1 Test portion

7.1.1 Liquid homogeneous products

Using a pipette, take 10 ml of the test sample (clause 6).

7.1.2 Liquid viscous products, and inhomogeneous, pasty, solid and dehydrated products

Weigh, to the nearest 0,01 g, 1 g to 10 g of the test sample (clause 6), according to the nature of the product.

7.2 Decomposition

Decomposition may be carried out using the dry or the wet method.

7.2.1 Decomposition using the dry method

7.2.1.1 Put the test portion (7.1) into a dish (5.3) and then proceed in accordance with either 7.2.1.2 or 7.2.1.3.

7.2.1.2 Place the dish on a water bath (5.7) set at 20 °C. Bring the temperature of the water progressively to boiling point (in order to avoid loss by too rapid heating), then evaporate to dryness.

Continue the decomposition in a muffle furnace (5.8) set at 525 °C.

7.2.1.3 If the electrical muffle furnace (5.8) can be temperature programmed in progressive steps from 20 °C to 525 °C, it is preferable to place the dish directly in the programmed electrical muffle furnace.

NOTE 1 It is preferable to dry the test portion by means of a temperature-programmed muffle furnace rather than by evaporation on a water bath in order to avoid loss due to too rapid heating.

7.2.1.4 If carbonized particles persist, add a few drops of the nitric acid (4.2), evaporate on a boiling water bath (5.7) [or in the furnace (5.8) at a temperature of less than 100 °C] then heat in the muffle furnace to 525 °C until the resulting ash has turned white.

7.2.1.5 Dissolve the ash in approximately 1 ml to 2 ml of the hydrochloric acid solution (4.4).

NOTE 2 This dissolution assists the transformation of mineral salts into easily dissociable chlorides.

Transfer quantitatively the ash solution to a centrifuge tube (5.6), rinsing the dish with approximately 20 ml of the hydrochloric acid solution (4.4), then centrifuge.

Remove the supernatant liquid and place it in a 50 ml volumetric flask (5.4). Dilute the residue in the centrifuge tube using 10 ml of the hydrochloric acid solution (4.4), centrifuge once again, remove the supernatant liquid and add it to the volumetric flask.

Dilute the residue in the centrifuge tube using 10 ml of water, centrifuge, remove the supernatant liquid and add it to the volumetric flask. Make up to the mark with water and mix the solution.

7.2.2 Decomposition using the wet method

Put the test portion (7.1) into a round-bottom flask of capacity appropriate for the size of the test portion. If the test portion contains ethanol, remove the ethanol by evaporation. Add 5 ml of the nitric acid (4.2), heat, then add carefully 5 ml of the sulfuric acid (4.1)[1]. Then proceed as described in ISO 5515:1979, subclause 6.3.1, second to eighth paragraphs.

As soon as the decomposition is complete, dilute the sulfuric acid solution with a few millilitres of water. Transfer quantitatively the solution to a centrifuge tube (5.6), rinsing the flask with about 10 ml of water and collecting the rinsings in the centrifuge tube. Centrifuge if necessary, then remove the supernatant liquid and place it in a 50 ml volumetric flask (5.4). Dilute the residue in the centrifuge tube using 10 ml of water, centrifuge once again, remove the supernatant liquid and add it to the volumetric flask. Repeat the dilution and centrifuging procedure with another 10 ml of water. Cool the solution in the volumetric flask, make up to the mark with water and mix.

7.2.3 Blank test

Prepare a blank solution using the same decomposition procedure (7.2.1 or 7.2.2), but replacing the test portion (7.1) with 10 ml of water.

7.3 Determination

7.3.1 Test portion decomposed using the dry method

7.3.1.1 Preparation of calibration graph

Dilute the standard copper solution (4.5) with the hydrochloric acid solution (4.4) to obtain five solutions with a copper content of 0,2 mg/l, 0,4 mg/l, 0,6 mg/l, 0,8 mg/l and 1 mg/l respectively.

Aspirate each of the calibration solutions, in turn, into the flame of the spectrometer (5.10), at a rate such that the maximum absorbance value is obtained for the solution having a copper content of 1 mg/l.

Take care to keep the aspiration rate constant throughout the preparation of the calibration graph. Spray water through the burner after each measurement.

Record the corresponding values of absorbance and plot the calibration graph.

7.3.1.2 Spectrometric measurements

Aspirate into the flame of the spectrometer (5.10), at the same aspiration rate as used in 7.3.1.1, the test solution obtained in 7.2.1 and the blank solution obtained in 7.2.3. Record the corresponding absorbances.

The absorbance of the blank solution shall be less than or equal to 0,002.

If the absorbance of the test solution is greater than that of the most concentrated solution used to prepare the calibration graph, dilute the test solution with the hydrochloric acid solution (4.4) as necessary and measure the absorbance.

7.3.2 Test portion decomposed using the wet method

7.3.2.1 Preparation of calibration graph

Dilute the standard copper solution (4.5) with water to obtain five solutions with a copper content of 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l respectively.

Into a series of five 50 ml one-mark volumetric flasks (5.4), place 5 ml of each of these solutions (one dilution per volumetric flask). Add 30 ml to 35 ml of

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[1] For certain products, 10 ml of sulfuric acid may be used, in which case the concentrations of sulfuric acid used in the preparation of the calibration curve (7.3.2.1) should be modified accordingly.

water and then 5 ml of the sulfuric acid (4.1) to each flask. Mix, allow to cool, and then make up to the mark with water and mix again.

The copper content of these solutions is 0,2 mg/l, 0,4 mg/l, 0,6 mg/l, 0,8 mg/l and 1 mg/l respectively.

Aspirate each of these solutions, in turn, into the flame of the spectrometer (5.10), at a rate such that the maximum absorbance value is obtained for the solution having a copper content of 1 mg/l.

Take care to keep the aspiration rate constant throughout the preparation of the calibration graph. Spray water through the burner after each measurement.

Record the corresponding values of absorbance and plot the calibration graph.

7.3.2.2 Spectrometric measurements

Aspirate into the flame of the spectrometer (5.10), at the same aspiration rate as used in 7.3.2.1, the test solution obtained in 7.2.2 and the blank solution obtained in 7.2.3. Record the corresponding absorbances.

The absorbance of the blank solution shall be less than or equal to 0,002.

If the absorbance of the test solution is greater than that of the most concentrated solution used to prepare the calibration graph, dilute the test solution with 10 % (*V/V*) sulfuric acid as necessary and measure the absorbance.

8 Calculation

8.1 Liquid homogeneous products

The copper content of the sample, expressed in milligrams per litre of product, is equal to

$$(c_1 - c_2) \times 5$$

where

c_1 is the copper content of the test solution, expressed in milligrams per litre, read from the calibration graph;

c_2 is the copper content of the blank solution, expressed in milligrams per litre, read from the calibration graph.

If the test solution was diluted, take the dilution factor into account in the calculation.

If it is desired to express the copper content of the dry product, take the moisture content of the sample into account in the calculation.

8.2 Liquid viscous products, and inhomogeneous, pasty, solid and dehydrated products

The copper content of the sample, expressed in milligrams per kilogram of product, is equal to

$$\frac{(c_1 - c_2)}{m_0} \times 50$$

where

c_1 is the copper content of the test solution, expressed in milligrams per litre, read from the calibration graph;

c_2 is the copper content of the blank solution, expressed in milligrams per litre, read from the calibration graph;

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

If the test solution was diluted, take the dilution factor into account in the calculation.

If it is desired to express the copper content of the dry product, take the moisture content of the sample into account in the calculation.

9 Repeatability

The absolute difference between two independent test results, obtained using 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, should not be greater than 10 % of the arithmetic mean of the two results.

10 Test report

The test report shall specify:

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the test result(s) obtained, and

— if the repeatability has been checked, the final quoted result obtained.