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Fruits, vegetables and derived products — Determination of iron content — 1,10-Phenanthroline photometric method

Fruits, légumes et produits dérivés — Détermination de la teneur en fer — Methode photométrique à la phénanthroline-1,10

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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.

International Standard ISO 5517 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1977.

It has been approved by the member bodies of the following countries :

Australia	Ghana	Poland
Austria	Hungary	Portugal
Bulgaria	India	Romania
Canada	Iran	*South Africa, Rep. of*
Czechoslovakia	Israel	Spain
Egypt, Arab Rep. of	Korea, Rep. of	Thailand
France	Mexico	
Germany	New Zealand	

No member body expressed disapproval of the document.

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Fruits, vegetables and derived products — Determination of iron content — 1,10-Phenanthroline photometric method

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a 1,10-phenanthroline photometric method for the determination of the iron content of fruits, vegetables and derived products.

2 REFERENCES

ISO 5515, *Fruits, vegetables and derived products — Decomposition of organic matter — Wet method.*[1]

ISO 5516, *Fruits, vegetables and derived products — Decomposition of organic matter — Ashing method.*[1]

3 PRINCIPLE

Decomposition of the organic matter, followed by reduction of trivalent iron by hydroxylammonium chloride. Formation, in a buffered medium, of the stable iron(II)/1,10-phenanthroline complex. Photometric measurement of the red-coloured complex at a wavelength of 508 nm.

4 REAGENTS

All reagents used shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

4.1 Sulphuric acid, ρ_{20} 1,84 g/ml.

4.2 Nitric acid, ρ_{20} 1,32 g/ml.

4.3 Hydrochloric acid, ρ_{20} 1,18 g/ml.

4.4 Hydroxylammonium chloride ($NH_2OH.HCl$), 200 g/l solution.

4.5 Buffer solutions.

4.5.1 Sodium acetate trihydrate ($NaCH_3CO_2.3H_2O$), 450 g/l solution.

4.5.2 Sodium acetate trihydrate ($NaCH_3CO_2.3H_2O$), 272 g/l (2 M) solution.

4.6 1,10-Phenanthroline, 10 g/l solution.

Dissolve 1 g of 1,10-phenanthroline in 80 ml of water at 80 °C and a minimum volume of the hydrochloric acid (4.3) diluted with an equal volume of water, in a 100 ml one-mark volumetric flask.

After cooling, dilute to the mark and mix.

This solution, stored in a cool place and protected from light, is stable for several weeks.

NOTE — Instead of 1,10-phenanthroline, the corresponding quantity of phenanthroline hydrochloride, which is soluble in cold water, may be used.

4.7 Iron, 0,020 g/l standard solution, prepared according to one of the two following procedures :

a) Weigh, to the nearest 0,001 g, 7,024 g of ammonium iron(II) sulphate hexahydrate [$(NH_4)_2Fe(SO_4)_2.6H_2O$]. Dissolve in water and add 2 drops of the hydrochloric acid (4.3). Transfer quantitatively to a 500 ml one-mark volumetric flask, dilute to the mark with water and mix. Using a pipette, transfer 10 ml of this solution to a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

b) Weigh, to the nearest 0,001 g, 0,200 g of iron wire of analytical purity. Dissolve in 200 ml of the hydrochloric acid (4.3) and add 50 ml of water. Transfer quantitatively to a 1 000 ml one-mark volumetric flask, dilute to the mark and mix. Using a pipette, transfer 50 ml of this solution to a 500 ml one-mark volumetric flask. Dilute to the mark with water and mix.

4.8 Magnesium acetate [$Mg(CH_3CO_2)_2$], 150 g/l solution.

1) At present at the stage of draft.

5 APPARATUS

Ordinary laboratory apparatus and

5.1 Kjeldahl flask, of capacity 250 or 300 ml.

5.2 Pipettes, of capacity 5, 10 and 20 ml, complying with class A of ISO 648.

5.3 Burettes, of capacity 50 ml, graduated in 0,1 ml, complying with class A of ISO/R 385.

5.4 One-mark volumetric flasks, of capacity 50 and 100 ml, complying with class A of ISO 1042.

5.5 Beaker, of capacity 50 ml.

5.6 Spectrophotometer or **photoelectric absorptiometer** suitable for measurements at a wavelength of 508 nm.

5.7 Analytical balance.

5.8 pH meter.

6 PROCEDURE

6.1 Preparation of test sample and test portion

See ISO 5515 or ISO 5516, according to the method of decomposition chosen (6.2). Take as the test portion about 10 g of the test sample, weighed to the nearest 0,001 g, or take 10 ml of the test sample using a pipette (5.2).

6.2 Decomposition

Proceed according to ISO 5515 or ISO 5516, diluting the test solution to 100 ml. (Note that if the decomposition is carried out according to ISO 5516, the dissolution of the ash is carried out after moistening it with 5 ml of sulphuric acid instead of 1 ml.)

6.3 Preliminary test

Carry out a preliminary test to determine the volume of buffer solution (4.5.1) to be added. According to the expected iron content, take, using a pipette (5.2), a volume V_1 ml (5, 10 or 20 ml) of the test solution obtained in 6.2.

Transfer to the 50 ml beaker (5.5), if necessary make up the volume to 20 ml with water, and then add 5 ml of the hydroxylammonium chloride solution (4.4).

Transfer to the beaker the volume of buffer solution (4.5.1) required to obtain a reading on the pH meter (5.8) between 3,5 and 4,5. Let X ml be the volume of buffer solution added.

6.4 Determination

According to the expected iron content, take a volume V_1 ml (see 6.3) of the test solution obtained in 6.2 and transfer to a 50 ml one-mark volumetric flask (5.4). If *necessary, make up the volume to 20 ml with water.*

Add 5 ml of the hydroxylammonium chloride solution (4.4) and X ml (see 6.3) of the buffer solution (4.5.1) in order to obtain a pH between 3,5 and 4,5*.

Add 2 ml of the 1,10-phenanthroline solution (4.6), dilute to the mark with water and mix. Allow to stand for 5 min.

Measure the absorbance using the spectrophotometer or the photoelectric absorptiometer (5.6) at a wavelength of 508 *nm. If the coloration is too strong, begin again, taking* a smaller volume V_1 or, if this is not possible, a smaller test portion.

6.5 Number of determinations

Carry out two determinations on the same test sample (6.1).

6.6 Blank test

Carry out a blank test, following the same procedure and using the same quantities of all the reagents as used for the determination but omitting the test portion.

6.7 Preparation of the calibration curve

Into a series of seven 100 ml one-mark volumetric flasks (5.4), *introduce respectively* 0 – 5 – 10 – 20 – 30 – 40 and 50 ml of the standard iron solution (4.7) and 2 ml of the hydrochloric acid (4.3). Dilute to the mark and mix. Then, into a series of seven 50 ml one-mark volumetric flasks (5.4), introduce 20 ml of each of the preceding solutions, corresponding respectively to 0 – 20 – 40 – 80 – 120 – 160 and 200 μg of iron. Add 5 ml of the hydroxylammonium chloride solution (4.4). Shake. Add 3,5 ml of the buffer solution (4.5.2). Shake. Add 2 ml of the 1,10-phenanthroline solution (4.6). Dilute to the mark and mix. Allow to stand for 5 min. Shake.

Measure the absorbance using the spectrophotometer or the photoelectric absorptiometer (5.6) at a wavelength of 508 nm. Subtract from the values found the absorbance corresponding to the blank test (6.6). Plot the calibration curve showing the number of micrograms of iron as a function of the absorbance.

* Although coloration develops at a pH between 2 and 9, its intensity is constant only between pH 3,5 and 4,5.

7 EXPRESSION OF RESULTS

7.1 Method of calculation and formulae

7.1.1 *Test portion taken by pipetting*

The iron content, expressed in milligrams per litre of product, is given by the formula

$$\frac{m_1}{1\,000} \times \frac{100}{V_1} \times \frac{1\,000}{V_0} = \frac{m_1 \times 100}{V_1 \times V_0}$$

where

m_1 is the mass, in micrograms, of iron read on the calibration curve (6.7);

V_0 is the volume, in millilitres, of the test portion (6.1);

V_1 is the volume, in millilitres, of the final portion taken for the determination (6.4).

7.1.2 *Test portion taken by weighing*

The iron content, expressed in milligrams per kilogram of product, is given by the formula

$$\frac{m_1}{1\,000} \times \frac{100}{V_1} \times \frac{1\,000}{m_0} = \frac{m_1 \times 100}{V_1 \times m_0}$$

where

m_0 is the mass, in grams, of the test portion (6.1);

m_1 is the mass, in micrograms, of iron read on the calibration curve (6.7);

V_1 is the volume, in millilitres, of the final portion taken for the determination (6.4).

7.2 Repeatability

The difference between the results of the two determinations carried out simultaneously or in rapid succession by the same analyst, on the same test sample, shall not exceed ± 3 % of the mean value.

8 TEST REPORT

The test report shall indicate the method used and the results obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances which may have influenced the results.

The test report shall give all details required for the complete identification of the sample.