International Standard ISO 6560

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Fruit and vegetable products — Determination of benzoic acid content (benzoic acid contents greater than 200 mg per litre or per kilogram) — Molecular absorption spectrometric method

Produits dérivés des fruits et légumes — Détermination de la teneur en acide benzoïque (teneurs supérieures à 200 mg par litre ou par kilogramme) — Méthode par spectrométrie 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.

International Standard ISO 6560 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in September 1982.

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

Australia	Iran	Poland
Austria	Iraq	South Africa, Rep. of
Canada	Ireland	Spain
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No member body expressed disapproval of the document.

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Fruit and vegetable products — Determination of benzoic acid content (benzoic acid contents greater than 200 mg per litre or per kilogram) — Molecular absorption spectrometric method

1 Scope and field of application

This International Standard specifies a method for the determination of the benzoic acid content of fruit and vegetable products.

The method is applicable to products having benzoic acid contents greater than 200 mg per litre or per kilogram and, in particular, to fruit juices, tomato purée, whether salted or not, and to products preserved with vinegar or lactic acid.

A method for the determination of benzoic acid at lower concentrations is specified in ISO 5518, *Fruits, vegetables and derived products — Determination of benzoic acid content — Spectrophotometric method.*

2 Principle

Extraction of the benzoic acid from an acidified test portion using diethyl ether, nitration followed by reduction, and Mohler's reaction modified with hydroxylamine hydrochloride. Spectrometric measurement of the absorbance of the red complex obtained.

3 Reagents

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

3.1 Benzoic acid, standard solution corresponding to 1 g of benzoic acid per litre.

Weigh, to the nearest 0,000 1 g, 100 mg of benzoic acid. Dissolve it in 25 ml of 0,1 mol/l sodium hydroxide solution and dilute to 100 ml with water.

1 ml of this standard solution contains 1 mg of benzoic acid.

3.2 Nitration solution.

Dissolve 23 g of potassium nitrate in 250 ml of concentrated sulfuric acid (ϱ_{20} = 1,84 g/ml).

3.3 Hydroxylamine hydrochloride, 20 g/l solution.

3.4 Ammonia solution, concentrated (ϱ_{20} = 0,910 g/ml).

3.5 Phenolphthalein solution.

Dissolve 1 g of phenolphthalein in 100 ml of 60 % (*V*/*V*) ethanol.

3.6 Sodium hydroxide, 1 mol/l solution.

3.7 Sodium hydroxide, 0,1 mol/l solution.

3.8 Sulfuric acid, 25 % (*m*/*m*) solution.

3.9 Carrez I solution.

Dissolve 150 g of potassium hexacyanoferrate(II) trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$] in water and dilute to 1 000 ml.

3.10 Carrez II solution.

Dissolve 300 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in water and dilute to 1 000 ml.

3.11 Diethyl ether, recently distilled.

4 Apparatus

Usual laboratory equipment, and in particular

4.1 Separating funnels, of capacity 200 ml, fitted with ground stoppers.

4.2 Pipettes, of capacities 1, 2, 5, 10, 25 and 50 ml.

4.3 Pipette, of capacity 2 ml, graduated in 0,1 ml divisions.

4.4 Volumetric flasks, of capacities 10 and 100 ml, fitted with a ground glass stopper.

4.5 Boiling water bath.

4.6 Test tubes, of diameter 1,5 or 2,0 cm.

4.7 Oven, capable of being controlled at 103 ± 2 °C.

4.8 Water bath, capable of being controlled at 20 ± 2 °C.

4.9 Water bath, capable of being controlled at 60 ± 2 °C.

4.10 Spectrometer, suitable for making measurements at a wavelength of 533 nm, with cells of optical path length 10 mm.

5 Procedure

5.1 Preparation of the test sample

5.1.1 Liquid products

Thoroughly mix the laboratory sample.

5.1.2 Semi-thick products (purée, etc.)

Thoroughly mix the laboratory sample. Press a part of the sample through four thickness of gauze, reject the first few drops of liquid and use the remainder for the determination. (Follow the instructions given for liquid products.)

5.1.3 Thick products

Thoroughly mix the laboratory sample.

5.2 Test portion

5.2.1 Liquid products

Take, by means of a pipette (4.2), 10 ml of the test sample and transfer it to a 200 ml separating funnel (4.1).

5.2.2 Thick products

5.2.2.1 Weigh, to the nearest 0,01 g, 10 g of the test sample.

5.2.2.2 Clarify the test portion as follows.

Add a little water to the test portion and make alkaline by adding the sodium hydroxide solution (3.6) in the presence of the phenolphthalein solution (3.5). Place on the boiling water bath (4.5) for 30 min. After cooling, transfer to a 100 ml volumetric flask, add 2 ml of the Carrez I solution (3.9) and 2 ml of the Carrez II solution (3.10), and dilute to the mark with water. Leave for 30 min and then centrifuge or filter. Use this clarified solution for the extraction (5.3).

5.3 Extraction and purification of the benzoic acid

5.3.1 Liquid products

Add 2 ml of the sulfuric acid solution (3.8) to the test portion in the separating funnel. Add 25 ml of the diethyl ether (3.11), shake, and allow the ethereal phase to separate. Repeat this extraction by shaking once more with 25 ml of the diethyl ether. Combine the ethereal phases.

Extract the benzoic acid from the ethereal phases, without washing with water, by adding 2 ml of the sodium hydroxide solution (3.6) in the presence of 1 drop of the phenolphthalein solution (3.5) and by shaking for 5 min. Separate the alkaline phase. Then extract twice by shaking with 2 ml of the sodium hydroxide solution (3.7). Collect the alkaline extracts in the 10 ml volumetric flask (4.4) and dilute to the mark with water.

5.3.2 Thick products

Add 50 ml of the sulfuric acid solution (3.8) to 50 ml of the solution obtained after clarification (5.2.2.2). Add 50 ml of the diethyl ether (3.11), shake, and allow the ethereal phase to separate. Repeat this extraction twice more by shaking with 50 ml of the diethyl ether. Combine the ethereal phases in a 200 ml separating funnel (4.1).

Add 5 ml of water, shake, and reject the aqueous phase.

Add 2 ml of the sodium hydroxide solution (3.6) to the ethereal phases and shake (sodium benzoate is thus formed). Separate the alkaline phase. Then extract twice by shaking with 2 ml of the sodium hydroxide solution (3.7). Collect the alkaline extracts in the 10 ml volumetric flask (4.4) and dilute to the mark with water.

5.4 Determination

5.4.1 Colour development

According to the expected benzoic acid content, transfer 0,5 to 1,0 ml of the alkaline extract to a test tube (4.6) and evaporate to dryness on the boiling water bath (4.5). Transfer to the oven (4.7) and dry for 15 min at 103 ± 2 °C.

Allow to cool, add 1 ml of the nitration solution (3.2) and place on the boiling water bath for 20 min. (Shaking the test tube during the first few minutes is recommended.)

Transfer to the water bath (4.8), maintained at 20 ± 2 °C, and leave for 15 min. Carefully add 2 ml of water and leave for a further 15 min. Add 10 ml of the ammonia solution (3.4), adding the first 5 ml in 0,5 ml fractions and the remainder in 1 ml fractions. Leave on the water bath for 15 min, taking care that the temperature of the water bath does not exceed 20 ± 2 °C. Add 2 ml of the hydroxylamine hydrochloride solution (3.3) and place on the water bath (4.9), maintained at 60 ± 2 °C, for 5 min.

5.4.2 Spectrometric measurement

Cool the test tube, fill a spectrometric cell with the solution and measure the absorbance at 533 nm in the following 30 min by means of the spectrometer (4.10).

5.5 Preparation of the calibration graph

5.5.1 Preparation of standard matching solutions

Into a series of five test tubes, transfer, by means of the pipette (4.3), the volumes of the standard benzoic acid solution (3.1) indicated in the table.

Table

Volume of standard benzoic acid solution (3.1)	Corresponding mass of benzoic acid
ml	mg
0,6	0,6
0,8	0,8
1,0	1,0
1,2	1,2
1,4	1,4

5.5.2 Colour development

Transfer the tubes to the boiling water bath (4.5) and evaporate to dryness. Then transfer the tubes to the oven (4.7) and dry for 15 min at 103 ± 2 °C.

Continue as described in 5.4.1, from the second paragraph onwards ["Allow to cool, add 1 ml of the nitration solution (3.2)..."].

5.5.3 Spectrometric measurements

Proceed as specified in 5.4.2.

5.5.4 Plotting the graph

Plot a graph having, for example, the masses, in micrograms, of benzoic acid in the standard matching solutions as abscissae and the corresponding values of absorbance as ordinates.

5.6 Number of determinations

Carry out two determinations on test portions taken from the same test sample (5.1).

6 Expression of results

6.1 Method of calculation and formulae

6.1.1 Liquid products

The benzoic acid content, expressed in milligrams per litre, is equal to

$$\frac{m \times V_2}{V_1 \times V_3} \times 1\,000$$

where

m is the mass, in milligrams, of benzoic acid, read from the calibration graph;

V_1 is the volume, in millilitres, of the test portion, i.e. 10 ml (see 5.2.1);

V_2 is the volume, in millilitres, of the alkaline extract, i.e. 10 ml (see 5.3);

V_3 is the volume, in millilitres, of alkaline extract taken for colour development (see 5.4.1).

6.1.2 Thick products

The benzoic acid content, expressed in milligrams per kilogram, is equal to

$$\frac{m_2 \times V_1 \times V_3}{m_1 \times V_2 \times V_4} \times 1\,000$$

where

m_1 is the mass, in grams, of the test portion;

m_2 is the mass, in milligrams, of benzoic acid, read from the calibration graph;

V_1 is the volume, in millilitres, of clarified solution prepared (see 5.2.2);

V_2 is the volume, in millilitres, of clarified solution taken for the extraction, i.e. 50 ml (see 5.3.2);

V_3 is the volume, in millilitres, of the alkaline extract, i.e. 10 ml (see 5.3);

V_4 is the volume, in millilitres, of alkaline extract taken for colour development (see 5.4.1).

6.2 Repeatability

The difference between the values obtained in the two determinations (5.6), carried out in rapid succession by the same analyst, shall not exceed 10 mg of benzoic acid per litre or per kilogram of product.

7 Note on procedure

The presence of formic acid and sorbic acid does not interfere with the determination of benzoic acid.

8 Test report

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

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