



SLOVENSKI STANDARD

SIST ISO 5518:2011

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Nadomešča:
SIST ISO 5518:1995

**Sadje, zelenjava in sadni ter zelenjavni proizvodi - Določevanje benzojske kisline -
Spektrofotometrična metoda**

Fruits, vegetables and derived products -- Determination of benzoic acid content --
Spectrophotometric method

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Fruits, légumes et produits dérivés -- Détermination de la teneur en acide benzoïque --
Méthode spectrophotométrique

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Sadje, zelenjava in njuni
proizvodi na splošno

Fruits, vegetables and
derived products in general

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en

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INTERNATIONAL STANDARD

ISO
5518

Second edition
2007-02-01

Fruits, vegetables and derived products — Determination of benzoic acid content — Spectrophotometric method

*Fruits, légumes et produits dérivés — Détermination de la teneur en
acide benzoïque — Méthode spectrophotométrique*

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Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 5518 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 3, *Fruit and vegetable products*.

This second edition cancels and replaces the first edition (ISO 5518:1978), which has been technically revised.

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Introduction

A method for determining the benzoic acid content of fruits, vegetable and derivatives, described in 1959, was based on the technique of peak emergence. The advantage of this method was that it was specifically intended for benzoic acid, with one exception: *p*-chlorobenzoic acid.

However, it was necessary to make a modification, consisting of purifying the ethereal extract by chromic acid oxidation. This results in the elimination of the effects of colouring substances in certain vegetable products containing anthocyanins, and also all the oxybenzoic acids and sorbic acid which may be present if several antiseptics have been used. Moreover, purification increases the sensitivity of the method. This improved technique was also more rapid.

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Fruits, vegetables and derived products — Determination of benzoic acid content — Spectrophotometric method

1 Scope

This International Standard specifies a method for determining the benzoic acid content of fruits, vegetables and derived products.

As chlorobenzoic acids are resistant to oxidation, the method cannot be applied in the presence of *p*-chlorobenzoic acid, as the absorption spectrum of this acid is close to that of benzoic acid. Neither can it be used in the presence of cinnamic acid, which is transformed into benzoic acid by chromic acid oxidation.

NOTE The cinnamic acid determined as benzoic acid in this method exists generally only in the form of traces in vegetables, and therefore has no effect on the result obtained, except in the case of cinnamon bark, which contains higher quantities.

2 Principle

The test sample is homogenized, followed by dilution and acidification of a test portion. The benzoic acid is extracted by diethyl ether, then this acid undergoes alkaline re-extraction and purification by oxidation using acidified potassium dichromate. The purified benzoic acid dissolved in diethyl ether is determined by spectrophotometry.

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3 Reagents

Use only reagents of recognized analytical quality and distilled water or water of at least equivalent purity.

3.1 Tartaric acid [COOH(CHOH)₂COOH], crystalline.

3.2 Sodium hydroxide (NaOH), approximately 1 mol/l solution.

3.3 Potassium dichromate (K₂Cr₂O₇), solution containing 33 g/l to 34 g/l.

3.4 Dilute sulfuric acid (H₂SO₄), obtained by diluting 2 volumes of concentrated sulfuric acid ($\rho_{20} = 1,84$ g/ml) with 1 volume of water.

3.5 Diethyl ether [(CH₃CH₂)₂O], recently distilled.

3.6 Benzoic acid (C₆H₅COOH), standard solution in diethyl ether containing 0,100 g/l.

3.7 Sodium hydrogen carbonate (NaHCO₃), crystalline.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Volumetric flasks, of capacities 50 ml and 1 000 ml.

4.2 Beakers, of capacities 50 ml and 100 ml.

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4.3 Graduated pipettes, of capacities 10 ml, 20 ml and 50 ml.

4.4 Flasks, of capacity 250 ml, having ground-glass stoppers and made of borosilicate glass.

4.5 Separating funnels, of capacities 100 ml and 500 ml.

4.6 Evaporating dish, of diameter about 10 cm.

4.7 Water bath, capable of being controlled at a temperature of 70 °C to 80 °C.

4.8 Homogenizer or mortar, as appropriate.

4.9 Spectrophotometer for determination in the ultraviolet range, equipped with a monochromator allowing measurement to the nearest 0,5 nm, with silica cells of optical path length 10 mm or 20 mm (preferably 20 mm so as to increase sensitivity), equipped with ground glass covers.

4.10 Analytical balance.

5 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

6 Procedure

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6.1 Preparation of test sample

6.1.1 Liquid products (e.g. juices, pulpy fluid products, syrups)
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Thoroughly mix the laboratory sample.

6.1.2 Thick products (e.g. marmalades, jams)

Homogenize the laboratory sample after having carefully mixed it.

6.1.3 Solid products (e.g. fruits, vegetables)

Cut a part of the laboratory sample into small pieces and remove seeds, stalks and carpellary cells, if necessary. Carefully homogenize approximately 40 g of the sample.

6.1.4 Frozen or deep-frozen products

After thawing the sample in a closed container and removing, if necessary, seeds, stalks and carpellary cells, mix the product with the liquid formed during the thawing process and proceed as described in 6.2.1, 6.2.2 or 6.2.3 as appropriate.

6.2 Preparation of test portion

6.2.1 Liquid products

Using a pipette (4.3), take 20 ml of the test sample (6.1), free from substances in suspension, dilute it with approximately 50 ml of water and transfer it to a 500 ml separating funnel (4.5) (separating funnel A).

The test portion may also be taken by mass by weighing, to the nearest 0,01 g, approximately 20 g of the test sample.

6.2.2 Pulpy and fluid products

Take 20 ml of the test sample (6.1). Place in a mortar (4.8) and dilute with 20 ml of water. After decanting, filter the liquid.

Twice successively, take up the residue in 20 ml of water and filter after decanting.

Collect all the filtrates directly in a 500 ml separating funnel (4.5) (separating funnel A).

The test portion may also be taken by mass by weighing, to the nearest 0,01 g, approximately 20 g of the test sample.

6.2.3 Thick or solid products

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample (6.1) and, using 30 ml to 40 ml of water, transfer it to a 250 ml flask (4.4).

Add approximately 50 mg of sodium hydrogen carbonate (3.7) (see Note). Shake, then place the flask on the water bath (4.7), controlled at 70 °C to 80 °C, and leave for 15 min to 30 min. Filter the contents of the flask and rinse twice using 15 ml to 20 ml of water each time.

Collect all the filtrates in a 500 ml separating funnel (4.5) (separating funnel A). Allow to cool.

NOTE The addition of sodium hydrogen carbonate is intended to neutralize the benzoic acid, traces of which could be lost by volatilization.

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6.3 Extraction of the benzoic acid

WARNING — Attention is drawn to the hazard derived from the use of diethyl ether, which is a highly flammable, explosive and harmful substance.

6.3.1 Introduce 1 g of the tartaric acid (3.1) into the separating funnel A (4.5) containing the diluted test portion (6.2), add 60 ml of the diethyl ether (3.5) and shake carefully.

Allow to separate, then collect the ethereal layer in a second 500 ml separating funnel (4.5) (separating funnel B).

Wash the aqueous phase in the first separating funnel (A) with 60 ml of the diethyl ether.

Allow to separate, then collect the ethereal layer in the separating funnel (B) containing the first layer collected.

Proceed similarly with a third extraction with 30 ml of the diethyl ether and combine the ethereal layer with the first two in the separating funnel (B).

6.3.2 Extract the benzoic acid from the ethereal solution by adding successively 10 ml and then 5 ml of the sodium hydroxide solution (3.2), and then twice 10 ml of water. After each addition, shake, then allow to separate and collect the aqueous phase.

Collect the aqueous phases in an evaporating dish (4.6). Place the dish on the water bath (4.7), controlled at 70 °C to 80 °C, and leave until the volume of the alkaline solution is reduced by approximately half, to remove the residual dissolved diethyl ether.