INTERNATIONAL STANDARD

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Fruit and vegetable products — Determination of benzoic acid and sorbic acid concentrations — High performance liquid chromatography method

Fruits, légumes et produits dérivés — Détermination des teneurs en acides benzoïque et sorbique — Méthode par chromatographie liquide à haute performance

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 22855 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 3, *Fruit and vegetable products*.

This corrected version of ISO 22855:2008 incorporates the following corrections:

— In 3.5 and 3.6, the concentrations of the stock solutions have been changed from 100 mg/ml to 1 g/l, and the texts have been reworded.

— The hyphen between high and performance in “high performance liquid chromatography” has been removed.

Fruit and vegetable products — Determination of benzoic acid and sorbic acid concentrations — High performance liquid chromatography method

1 Scope

This International Standard specifies a method using high performance liquid chromatography for the determination of the concentration of benzoic and sorbic acids in fruit and vegetable juices.

NOTE This method is based on IFU method 63 [2].

2 Principle

Extraction of benzoic acid and/or sorbic acid from a test portion using a mixture of ammonium acetate buffer solution and methanol, under acidic conditions.

The concentration of benzoic and/or sorbic acid is determined by means of high performance liquid chromatography (HPLC) using a reverse phase column and ultraviolet (UV) detector.

3 Reagents and materials

Use only reagents of recognized analytical grade, unless otherwise specified, and water of HPLC grade.

3.1 Acetic acid (CH_3COOH), glacial.

3.2 Methanol (CH_3OH), for HPLC.

3.3 Ammonium acetate (CH_3COONH_4), 0,01 mol/l solution.

Dissolve 0,771 g of ammonium acetate in 1 l of water.

3.4 Ammonium acetate/acetic acid (CH_3COONH_4/CH_3COOH), buffer solution.

Mix 1 000 volume parts of ammonium acetate solution (3.3) with 1,2 volume parts of acetic acid (3.1).

3.5 Benzoic acid (C_6H_5COOH), stock solution.

Dissolve 100 mg of benzoic acid in 40 ml of methanol (3.2) and make up to the mark with water in a 100 ml volumetric flask, to obtain the stock solution, $\rho(C_6H_5\,COOH)$ = 1 g/l.

3.6 Sorbic acid [$CH_3(CH{:}CH)_2COOH$], stock solution.

Dissolve 100 mg of sorbic acid in 40 ml of methanol (3.2) and make up to the mark with water in a 100 ml volumetric flask, to obtain the stock solution, $\rho[CH_3\,(CH{:}CH)_2\,COOH]$ = 1 g/l.

3.7 Potassium hexacyanoferrate(II), trihydrate, $K_4[Fe(CN)_6]{\cdot}3H_2O$.

3.8 Zinc sulfate, heptahydrate, ($ZnSO_4 \cdot 7H_2O$), 300 g/l solution.

3.9 Extraction solution

Mix 60 volume parts of ammonium acetate/acetic acid buffer solution (3.4) with 40 volume parts of methanol (3.2).

3.10 Eluent for HPLC

Mix 50 volume parts of ammonium acetate solution (3.4) with 40 volume parts of methanol for HPLC (3.2) and adjust to a pH of 4,5 to 4,6 with acetic acid (3.1). Filter the eluent over a membrane filter (4.2).

3.11 Carrez solution I

Dissolve 150 g of potassium hexacyanoferrate(II) (3.7) in water in a 1 000 ml volumetric flask. Dilute to the mark with water and mix the solution.

3.12 Carrez solution II

Dissolve 300 g of zinc sulfate (3.8) in water in a 100 ml volumetric flask. Dilute to the mark with water and mix the solution.

3.13 Pleated filter paper, hard.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Ultrasonic bath.

4.2 Membrane filters, of pore size 0,45 µm, for aqueous solutions (e.g. cellulose acetate); diameter dependent on the filter holder.

4.3 Filter holder, for membrane filters with suitable aspirating and collection vessels.

4.4 High performance liquid chromatograph, equipped with a UV-detector (variable wavelength) and recorder and/or integrator or computer with the appropriate integrating programme.

4.5 Reverse phase separation column, e.g. reverse phase C8, 250 mm × 4,6 mm, particle size 5 µm.

5 Sample

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

6 Procedure

6.1 Preparation of test solution

Homogenize or mix the sample carefully.

Concentrated juice should be diluted to single strength.

6.1.1 Clear samples

Dilute 5,00 ml to 10,00 ml (V_1) of a sample in approximately 75 ml of extraction solution (3.9) in a 100 ml volumetric flask. Put the flask in the ultrasonic bath (4.1), mix the contents for at least 10 min and then dilute to the mark with extraction solution (3.9) at 20 °C. Filter the solution through a membrane filter (4.2).

6.1.2 Cloudy samples

Dilute 5,00 ml to 10,00 ml (V_1) of a sample in approximately 75 ml of extraction solution (3.9) in a 100 ml volumetric flask. Put the flask in the ultrasonic bath (4.1) and mix the contents for at least 10 min. Then add 1,0 ml of Carrez solution I (3.11) and 1,0 ml of Carrez solution II (3.12) for clarification. Mix the solution carefully after each addition and dilute to the mark with the extraction solution (3.9) at 20 °C. Filter the solution over a paper filter (3.13); discard the first millilitres of filtrate. Filter the clear solution through a membrane filter (4.2).

6.2 Preparation of the calibration curves

Dilute benzoic acid stock solution (3.5) and/or sorbic acid stock solution (3.6) with extraction solution (3.9) at 20 °C to obtain standard solutions I, II and III with benzoic acid and/or sorbic acid concentrations of 10 mg/l, 25 mg/l and 50 mg/l, respectively.

Inject 10 µl of each of the calibration solutions into the chromatograph (4.4), under the following conditions:

— flow rate: approximately 1,2 ml/min;

— wavelength for UV-detection: 235 nm (0,08 AUFS - absorbance unit full scale).

Prepare the calibration curves by plotting the peak areas against benzoic acid and/or sorbic acid concentration, in milligrams per litre.

6.3 Determination

Inject 10 µl of the test solution (6.1) into the chromatograph using the same conditions as for the preparation of the calibration graph.

Identify the benzoic acid and/or sorbic acid peaks of the test solution by comparison with the peaks of the calibration solutions.

NOTE 1 For optimal separation of benzoic and/or sorbic acid, a slight change in the composition of the eluent may be necessary.

NOTE 2 Under the conditions described in this procedure, it is possible to determine the methyl, ethyl, and propyl esters of 4-hydroxybenzoic acid as well (see chromatogram in Annex A).

NOTE 3 Matrix peaks can cause interference with the analysis of benzoic acid in orange juice. In such a case, a suitable clean-up step is necessary.

NOTE 4 Identification of benzoic and sorbic acids in a sample is performed by comparing with the retention time of the standard solutions. It is possible to identify the analysed acids by using other methods of identification: spiking with single substances, viewing of absorption spectra at required wavelengths and measuring the absorption at different wavelengths.

NOTE 5 Quantification is carried by the external standard method with integration of peak area or measurement of peak heights. It is necessary to check linearity of the calibration function, e.g. with standard solutions I, II and III.

7 Calculation

Determine the concentration of benzoic and/or sorbic acid in the test solution directly from the calibration curve (6.2). Calculate the benzoic acid concentration of the sample, ρ_A in milligrams per litre, using the following equation (external standard method):

$$\rho_A = \frac{A_1 \cdot \rho_{st} \times 100}{A_2 \cdot V_1}$$

where

A_1 is the peak area or peak height of benzoic acid or sorbic acid in the test sample, expressed in area or length counts, respectively;

A_2 is the peak area or peak height of benzoic acid or sorbic acid in the test standard solution, expressed in area or length counts, respectively;

ρ_{st} is the concentration of the standard solution, in milligrams per litre;

V_1 is the volume of the test sample solution, in millilitres.

The result(s) is(are) expressed, in milligrams per litre, to one decimal place.

8 Precision

The precision of the method was established by an interlaboratory test organized by the analytical commission of the International Fruit Union (IFU). In this test, samples of orange and grape juices were investigated. The statistical treatment was performed by the Max von Pettenkofer Institut of the German Federal Office of Public Health. See Annex B for a summary of the statistical results of this test.

Statistical parameters are expressed in accordance with ISO 5725-2 [1].

8.1 Repeatability

Benzoic acid

Orange juice: r = 3,5 s_r = 1,25

Grape juice: r = 3,5 s_r = 1,25

Sorbic acid

Orange juice: r = 2,8 s_r = 1,00

Grape juice: r = 2,3 s_r = 0,88

4-Hydroxybenzoic acid methyl ester

Orange juice: r = 4,5 s_r = 1,60

Grape juice: r = 3,8 s_r = 1,37

4-Hydroxybenzoic acid ethyl ester

Orange juice: r = 4,5 s_r = 1,59

Grape juice: r = 4,8 s_r = 1,70

4-Hydroxybenzoic acid propyl ester

Orange juice: r = 5,3 s_r = 1,88

Grape juice: r = 5,4 s_r = 1,93

where

r is the repeatability limit;

s_r is the standard deviation of repeatability.

8.2 Reproducibility

Benzoic acid

Orange juice: R = 13,9 s_R = 4,96

Grape juice: R = 8,9 s_R = 3,18

Sorbic acid

Orange juice: R = 11,0 s_R = 3,93

Grape juice: R = 7,3 s_R = 2,61

4-Hydroxybenzoic acid methyl ester

Orange juice: R = 19,0 s_R = 6,79

Grape juice: R = 11,7 s_R = 4,17

4-Hydroxybenzoic acid ethyl ester

Orange juice: R = 9,8 s_R = 3,5

Grape juice: R = 8,8 s_R = 3,14

4-Hydroxybenzoic acid propyl ester

Orange juice: R = 16,8 s_R = 6,00

Grape juice: R = 17,7 s_R = 6,32

where

R is the reproducibility limit;

s_R is the standard deviation of reproducibility.

9 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

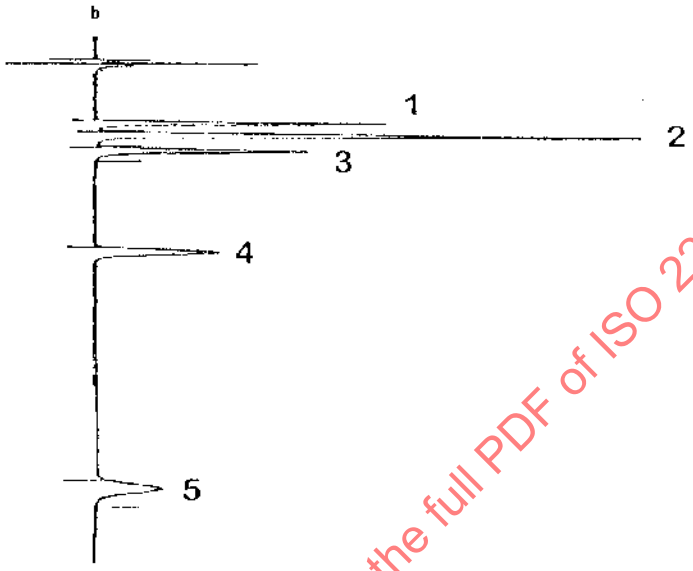
c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained, and, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Chromatogram



Column	Diameter 4,6 mm, length 250 mm	
	Ultrasphere-octyl (RP 8), particle size 5 µm	
Eluent	Ammonium acetate solution/Methanol for HPLC (50 + 40 volume parts)+ Acetic acid (pH = 4.5)	
Flow rate	1,2 ml/min	
UV-detection	235 nm (0,08 AUFS)	
Recorder speed	3 mm/min	
Injection volume	10 µl	
Standard substances:	1. Benzoic acid	(RT:8,13) [a]
	2. Sorbic acid	(RT:9,33)
	3. 4-Hydroxybenzoic acid methyl ester	(RT:10,86)
	4. 4-Hydroxybenzoic acid ethyl ester	(RT:20,34)
	5. 4-Hydroxybenzoic acid propyl ester	(RT:42,36)

[a] RT = Retention time, minutes.

[b] Inject.

ρ(standard) = 25 mg/l in each case

Figure A.1 — HPLC-separation of standard solution II (6.2)

Annex B
(informative)

Statistical results of the interlaboratory test

Table B.1 — Benzoic acid

Parameters	Sample	
	Orange juice	Grape juice
Mean benzoic acid concentration (mg/l)	72,8	57,0
Repeatability standard deviation s_r (mg/l)	1,25	1,25
Repeatability coefficient of variation CV_r (%)	1,71	2,19
Repeatability limit $r = 2{,}8 \times s_r$ (mg/l)	3,5	3,5
Reproducibility standard deviation s_R (mg/l)	4,96	3,18
Reproducibility coefficient of variation CV_R (%)	6,81	5,57
Reproducibility limit $R = 2{,}8 \times s_R$ (mg/l)	13,9	8,9

Table B.2 — Sorbic acid

Parameters	Sample	
	Orange juice	Grape juice
Mean sorbic acid concentration (mg/l)	91,1	65,7
Repeatability standard deviation s_r (mg/l)	1,0	0,88
Repeatability coefficient of variation CV_r (%)	1,10	1,34
Repeatability limit $r = 2{,}8 \times s_r$ (mg/l)	2,8	2,3
Reproducibility standard deviation s_R (mg/l)	6,79	2,61
Reproducibility coefficient of variation CV_R (%)	7,45	3,97
Reproducibility limit $R = 2{,}8 \times s_R$ (mg/l)	11,0	7,3

Table B.3 — 4-Hydroxybenzoic acid methyl ester

Parameters	Sample	
	Orange juice	Grape juice
Mean 4-hydroxybenzoic acid methyl ester concentration (mg/l)	83,4	75,5
Repeatability standard deviation s_r (mg/l)	1,60	1,37
Repeatability coefficient of variation CV_r (%)	1,92	1,81
Repeatability limit $r = 2{,}8 \times s_r$ (mg/l)	4,5	3,8
Reproducibility standard deviation s_R (mg/l)	6,79	4,17
Reproducibility coefficient of variation CV_R (%)	8,141	5,52
Reproducibility limit $R = 2{,}8 \times s_R$ (mg/l)	19,0	11,7