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Fruits, vegetables and derived products — Determination of sorbic acid content

Fruits, légumes et produits dérivés — Détermination de la teneur en acide sorbique

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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ISO 5519 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 5519:1978), which has been technically revised.

Introduction

The determination of the sorbic acid content of fruits, vegetables and derived products has been studied in numerous projects during the acid's use as a fungicide, especially in wines. Because of its great volatility (very similar to that of acetic acid), the simplest extraction process is its entrainment by steam. This method has the advantage of producing an almost pure aqueous solution of sorbic acid.

Two techniques for the determination of the quantity of sorbic acid contained in this solution are described in this International Standard, namely:

— Technique A: spectrophotometry in the ultraviolet range, carried out after oxidation of sulfur dioxide, which would interfere. The oxidation occurs spontaneously in a few minutes in air, after the addition of a trace of a copper catalyst.

— The natural essential oils of citrus fruits do not interfere with the determination, provided that they are present in the small quantities normal in juice not enriched with essential oils. When the quantities of essential oils are significant, they may be eliminated beforehand by the same method as that applied in technique B.

— Technique B: colorimetry based on Schmidt's reaction, which requires the elimination of ethanol and essential oils by the evaporation of an aliquot portion of the distillate. This technique, not as rapid as technique A, but giving comparable results, is provided for use when a spectrophotometer allowing measurements in the ultraviolet range is not available.

The interference caused by essential oils of garlic, onion or leek may be eliminated, when using either technique, by the evaporation of an aliquot portion of the distillate.

Fruits, vegetables and derived products — Determination of sorbic acid content

1 Scope

This International Standard specifies a method for extracting the sorbic acid present in fruits, vegetables and derived products, and two techniques for determining the sorbic acid extracted.

2 Principle

Homogenization of the product, followed by quantitative entrainment, by steam, of the sorbic acid present in a test portion. Determination of this acid in the distillate obtained, either by spectrophotometry in the ultraviolet range (technique A), or by measuring by photocolorimetry or by spectrophotometry, the pink colour obtained after oxidation by chromic acid and then treatment with thiobarbituric acid (technique B).

3 Reagents

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

3.1 Tartaric acid [COOH (CH OH)$_2$ COOH], crystalline.

3.2 Sorbic acid [$CH_3(CH{:}CH)_2COOH$], 0,010 g/l standard solution, prepared by one of the following methods (3.2.1 or 3.2.2).

3.2.1 Dissolve 0,100 g of sorbic acid in 10 ml to 12 ml of a 0,1 N sodium hydroxide solution. Transfer quantitatively into a 1 000 ml volumetric flask, and dilute to the mark with water. Introduce 100 ml of the solution obtained into a second 1 000 ml flask, and dilute to the mark with water.

3.2.2 Dissolve 0,134 g of potassium sorbate, $CH_3(CH{:}CH)_2COOK$, (previously recrystallized and dried to constant mass in a drier at 105 °C or in a desiccator over concentrated sulfuric acid) in water in a 1 000 ml volumetric flask, and dilute to the mark with water. Introduce 100 ml of the solution obtained into a second 1 000 ml flask, and dilute to the mark with water.

3.3 Calcium hydroxide [$Ca(OH)_2$] (if necessary), about 0,04 N (1,48 g/l) solution.

3.4 Acetic acid (CH_3COOH), 0,1 N solution.

3.5 Lactic acid [$CH_3CH(OH)COOH$], 1 N solution.

3.6 Copper, catalyst solution, for technique A.

In a 1 000 ml volumetric flask, dissolve, in a little water, 0,5 g of sodium hydrogen carbonate ($NaHCO_3$), and 0,001 g of pure copper(II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$). Dilute to the mark with water.

3.7 Chromic/sulfuric acid solution, for technique B.

Dissolve 0,050 g of potassium dichromate ($K_2Cr_2O_7$) in approximately 90 ml of water. Transfer quantitatively into a 200 ml volumetric flask. Add 100 ml of a 0,3 N sulfuric acid (H_2SO_4) solution. Dilute to the mark with water.

1 litre of 0,3 N sulfuric acid solution contains 14,7 g of sulfuric acid, i.e. 8,4 ml of sulfuric acid, $\rho_{20} = 1{,}84$ g/ml.

3.8 Thiobarbituric acid solution, for technique B.

Dissolve 0,500 g of thiobarbituric acid ($C_4H_4N_2O_2S$) in 50 ml of water to which has been added 10 ml of a 1 N sodium hydroxide (NaOH) solution. Transfer quantitatively into a 100 ml volumetric flask, and add 11 ml of a 1 N hydrochloric acid (HCl) solution. Dilute to the mark with water.

This solution is not stable and must be used within 5 h following its preparation.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Analytical balance.

4.2 Homogenizer or mortar, as appropriate.

4.3 Boiling water bath.

4.4 Steam distillation apparatus (see Figure A.1), comprising the items listed in 4.4.1 to 4.4.5.

4.4.1 Steam generator flask, of capacity 1 000 ml to 1 500 ml.

4.4.2 Bubbler, consisting of a cylindrical tube 30 mm in diameter and 270 mm in height, the lower part of which is closed and enlarged into a sphere having a diameter of 60 mm. The steam supply tube shall end 10 mm above the bottom of the bubbler. The spherical part, in which the product is placed, may be heated either electrically or by a flame. In the latter case, the burnt gases shall be deflected by a metal disc of 150 mm diameter, having a central orifice of approximately 40 mm diameter in which the bottom of the bubbler is engaged. This device avoids the pyrogenation of materials which may be extracted from the product. The auxiliary heating shall be controlled so that the volume of the product placed in the bubbler neither decreases nor increases by more than 5 ml during the distillation.

4.4.3 Fractionating column, through which the vapour containing volatile acid passes. It may consist of

— a cylindrical tube of diameter 20 mm and height 500 mm, containing a corrugated helix of No. 100 stainless steel mesh, the helix having a pitch of 15 mm, or

— a column of diameter 20 mm and height 600 mm, having glass internal points, or

— any other device having the same fractionating efficiency.

The fractionation of the vapour is indispensable to retain hydroxymethylfurfural when it is present. This substance and its hydrolysis products absorb ultraviolet radiations at 256 nm. The fractionating column may be reduced to 200 mm in height or be replaced by a Kjeldahl flask when the product is free of hydroxymethylfurfural.

4.4.4 Condenser, of the West type, of effective length 400 mm, placed vertically to ensure condensation of the vapour and complete cooling of the distillate.

4.4.5 Receiver flasks, of capacities 200 ml or 500 ml:

— liquid products: 200 ml flask with a graduation mark at 200 ml;

— thick or solid products: 500 ml flask.

4.4.6 Checking of efficiency of steam distillation apparatus.

The distillation apparatus shall allow 300 ml of distillate to be collected in 12 min to 15 min, and shall also comply with the following minimal conditions.

a) In normal distilling conditions, 99,5 % of a known quantity of acetic acid added to the sample shall be found in the distillate, which shall be 200 ml. For this test, use 20 ml of a 0,1 N acetic acid solution (3.4).

b) In the same distilling conditions, not more than 5 parts per thousand of a known quantity of lactic acid added to the sample shall be found in the distillate, which shall be 200 ml. For this test, use 20 ml of 1 N lactic acid solution (3.5).

4.5 Pipettes, of capacities 10 ml, 20 ml and 25 ml.

4.6 Graduated pipettes, of appropriate capacities.

4.7 Evaporating dish.

4.8 Conical flasks, for technique A, of capacity 50 ml.

4.9 Spectrophotometer, for technique A, allowing measurements at a wavelength of 256 nm (ultraviolet), with silica cells of 10 mm optical path length.

4.10 Volumetric flasks, for technique B, of capacity 25 ml.

4.11 Photocolorimeter, fitted with a green filter, or **spectrophotometer** allowing measurements at a wavelength of 532 nm, for technique B.

5 Sample

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

6 Procedure

6.1 Preparation of test sample

6.1.1 Liquid products (juices, pulpy fluid products, syrups, etc.)

Thoroughly mix the laboratory sample.

6.1.2 Thick products (marmalades, jams, etc.)

Homogenize the laboratory sample after having carefully mixed it.

6.1.3 Solid products (fruits, vegetables)

Cut a part of the laboratory sample into small pieces, and remove seeds, stalks and carpellary cells, if necessary, and 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 6.1.1, 6.1.2 or 6.1.3, as appropriate.

6.2 Test portion

6.2.1 Liquid products

Using a pipette (4.5), take 10 ml of the test sample (6.1) and introduce it into the bubbler (4.4.2).

The test portion may also be taken by mass, by weighing, to the nearest 0,01 g, approximately 10 g of the test sample.

6.2.2 Thick or solid products

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample (6.1) and introduce it into the bubbler (4.4.2) with the minimum of water necessary to entrain the whole of the test portion and to make the mixture sufficiently fluid.

NOTE In certain cases, it is necessary to leave the test portion to soak in the water for 1 h to 2 h.

6.3 Distillation

6.3.1 General

Introduce 0,5 g of the tartaric acid (3.1) into the bubbler (4.4.2) containing the test portion (6.2). Connect the bubbler to the steam generator flask (4.4.1) and to the condenser (4.4.4) and simultaneously heat the flask and the bubbler. Then carry out the distillation, making sure that the volume of the contents of the bubbler remains constant to within 5 ml.

6.3.2 In the case of liquid products (6.2.1)

Collect the distillate in the 200 ml receiver flask (4.4.5), stopping the distillation when the 200 ml mark is reached.

6.3.3 In the case of thick or solid products (6.2.2)

Collect, in the 500 ml flask (4.4.5), a volume of distillate at least 20 times greater than the volume of the contents of the bubbler. Measure the volume (V) collected, using a graduated cylinder.

6.4 Technique A: determination by spectrophotometry in the ultraviolet range

6.4.1 Determination

6.4.1.1 If the initial product contains essential oils of garlic, onion or leek, the presence of these essential oils causes significant absorbance, especially in the case of garlic. Complete evaporation[1)] of the distillate, after its being made alkaline, allows the effect of this absorbance to be counteracted.

When these essential oils are present, therefore, take, with a pipette (4.5), 25 ml distillate (6.3) and transfer it to a small evaporating dish (4.7); make it alkaline with 1,5 ml to 2 ml of the calcium hydroxide solution (3.3), evaporate it to dryness on the boiling water bath (4.3) and reconstitute it with water to re-establish the initial volume.

1) The evaporation to dryness does not destroy the sorbic acid if the conditions are sufficiently alkaline.

6.4.1.2 As appropriate, take with a pipette (4.5) 10 ml (see Note) of the distillate (6.3) or of the reconstituted solution (6.4.1.1), place it in a 50 ml conical flask (4.8), and add 10 ml of the copper catalyst solution (3.6). Shake briefly and leave to stand in contact with air for several minutes.

NOTE The volume of 10 ml is intended for products containing up to 200 mg of sorbic acid per litre or per kilogram. For higher contents, take only 5 ml or 2 ml and dilute to 10 ml with water.

Measure the absorbance of the solution using the spectrophotometer (4.9), at a wavelength of 256 nm.

Subtract, from the value found, the absorbance of the blank test solution (6.4.2).

6.4.2 Blank test

Carry out a blank test in parallel with the determination, replacing the 10 ml of distillate by 10 ml of water.

6.4.3 Number of determinations

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

6.4.4 Preparation of calibration curve

6.4.4.1 Into a series of six 50 ml conical flasks (4.8), introduce respectively, with a graduated pipette (4.6): 0 ml; 1 ml; 2 ml; 3 ml; 5 ml and 10 ml of the standard sorbic acid solution (3.2); make up the volume to 10 ml by adding water.

The solutions obtained contain: 0 mg; 1 mg; 2 mg; 3 mg; 5 mg and 10 mg of sorbic acid per litre.

6.4.4.2 To each flask, add 10 ml of the copper catalyst solution (3.6).

Measure the absorbances of the solution using the spectrophotometer (4.9) at a wavelength of 256 nml.

Subtract, from the values found, the absorbance of the blank test solution (6.4.2).

6.4.4.3 Plot the calibration curve, showing the absorbances of the solution (6.4.4.2) as a function of the sorbic acid concentrations of the solutions obtained in 6.4.4.1, i.e. before addition of the copper catalyst solution (3.6), expressed in milligrams per litre.

6.5 Technique B: determination by photocolorimetry or by spectrophotometry at 532 nm

6.5.1 Determination

6.5.1.1 If the initial product contains ethanol, remove it from the distillate by the following method.

Using the pipette (4.5) introduce 25 ml of the distillate (6.3) into a small evaporating dish (4.7), and make it alkaline with 1,5 ml to 2 ml of the calcium hydroxide solution (3.3), place the dish on the boiling water bath (4.3), and evaporate until the volume is reduced by about half, which normally takes about 30 min. Quantitatively transfer the residue into the 25 ml volumetric flask (4.10). Make up to the mark with rinsing water from the dish. Shake.

6.5.1.2 If the initial product contains essential oils (in the case of juice from citrus fruits), eliminate them from the distillate by the same method as described in 6.5.1.1, but prolong the evaporation so that a volume of 1 ml to 2 ml is attained.

6.5.1.3 If the initial product contains essential oils of garlic, onion or leek, proceed as indicated in 6.4.1.1.

NOTE In the case of garlic, even when the distillate is evaporated to dryness, a very weak absorbance, corresponding to 1,5 mg of sorbic acid per kilogram, remains.

6.5.1.4 As appropriate, take, with a pipette (4.5), 10 ml (see next paragraph) of the distillate (6.3) or of the reconstituted solution obtained after treatment (6.5.1.1, 6.5.1.2, or 6.5.1.3), and introduce it into the 25 ml volumetric flask (4.10).

The volume of 10 ml is intended for products containing up to 200 mg of sorbic acid per litre or per kilogram. For higher contents, take only 5 ml or 2 ml and dilute to 10 ml with water.

Add 4 ml of the chromic/sulfuric acid solution (3.7) and keep the flask for 10 min in the boiling water bath (4.3).

Add 4 ml of the thiobarbituric acid solution (3.8) and keep the flask for 10 min in the boiling water bath for a further 20 min; a pink colour will develop. Cool in an iced water bath and dilute to the mark with water.

Within 30 min, measure the absorbance of the solution using the photocolorimeter or spectrophotometer (4.11) at a wavelength of 532 nm.

Subtract, from the value found, the absorbance of the blank test solution (6.5.2).

6.5.2 Blank test

Carry out a blank test in parallel with the determination, replacing the 10 ml of distillate by 10 ml of water.

6.5.3 Number of determinations

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

6.5.4 Preparation of calibration curve

6.5.4.1 Prepare a 2,0 mg/l standard sorbic acid solution by diluting 1 volume of the standard solution (3.2) with 4 volumes of water.

6.5.4.2 Into a series of six 25 ml volumetric flasks (4.10), introduce respectively, with a graduated pipette (4.6): 0 ml; 2 ml; 4 ml; 6 ml; 8 ml and 10 ml of the diluted standard sorbic acid solution (6.5.4.1); make up the volume to 10 ml by adding water.

The solutions obtained contain: 0 mg; 0,4 mg; 0,8 mg; 1,2 mg; 1,6 mg and 2,0 mg of sorbic acid per litre.

6.5.4.3 To each flask add 4 ml of the chromic/sulfuric acid solution (3.7) and keep the flasks for 10 min in the boiling water bath (4.3).

Add 4 ml of the thiobarbituric acid solution (3.8) and keep the flasks for 10 min in the boiling water bath for a further 20 min; a pink colour will develop. Cool in an iced water bath and dilute to the mark with water.

Within 30 min, measure the absorbance of the solution using the photocolorimeter or spectrophotometer (4.11) at a wavelength of 532 nm.

Subtract, from the value found, the absorbance of the blank test solution (6.5.2).

6.5.4.4 Plot the calibration curve, showing the absorbance of the solutions (6.5.4.3) as a function of the corresponding concentration of sorbic acid, expressed in milligrams per litre.

7 Calculation and expression of results[2)]

7.1 Test portion measured by volume

The sorbic acid concentration, expressed in milligrams per litre of product, is given by the formula

$$\frac{m_1 \times 200}{V_1}$$

where

m_1 is the mass of sorbic acid, expressed in milligrams, per litre of distillate (6.5.1), read on the calibration curve (see 6.4.4 or 6.5.4);

V_1 is the volume, in millilitres, taken in 6.4.1.2 or 6.5.1.4 (usually 10 ml, but may be reduced to 5 ml or 2 ml).

7.2 Test portion measured by mass

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

$$\frac{m_1 \times V \times 10}{m_0 \times V_1}$$

where

m_0 is the mass, in grams, of the test portion (6.2.2);

m_1 is the mass of sorbic acid, expressed in milligrams, per litre of distillate (6.3.3), read on the calibration curve (see 6.4.4 or 6.5.4);

V is the volume, in millilitres, of distillate collected (see 6.3.3);

V_1 is the volume, in millilitres, taken in 6.4.1.2 or 6.5.1.4 (usually 10 ml, but may be reduced to 5 ml or 2 ml).

8 Repeatability

The absolute difference between two single test results, obtained with 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, will in not more than 5 % of cases be greater than 5 % the arithmetic mean of the two results.

2) Various vegetable products contain small quantities of volatile substances which may be extracted by organic solvents and which absorb 256 nm radiation or give the coloured reaction used in technique B (6.5). Therefore, results which are only slightly positive (less than 10 mg per litre or per kilogram) should be interpreted with caution and compared with results from the same products free from sorbic acid.

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;

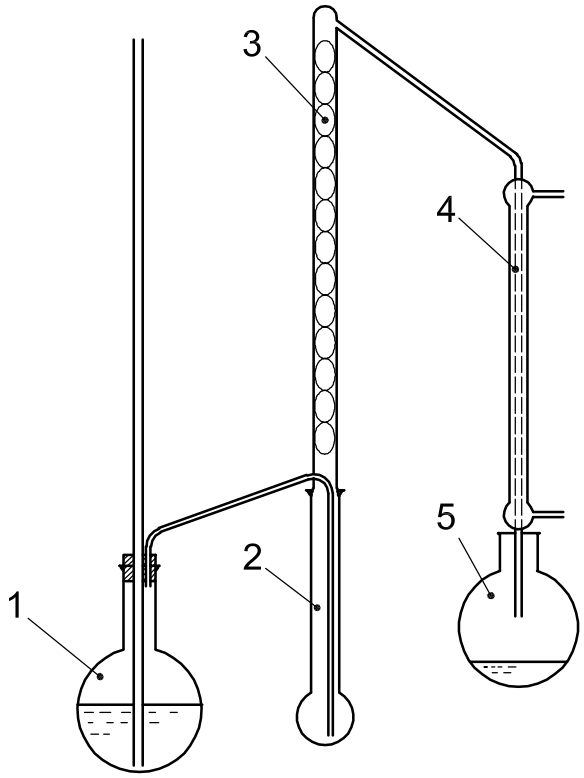
c) the test method used, with reference to this International Standard;

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

Annex A
(normative)

Steam distillation apparatus



Key

1 steam generator flask (4.4.1)
2 bubbler (4.4.2)
3 fractionating column (4.4.3)
4 condenser (4.4.4)
5 receiver flask (4.4.5)

Figure A.1 — Diagram of steam distillation apparatus (4.4)

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