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International Standard



6557/2

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**Fruits, vegetables and derived products — Determination  
of ascorbic acid content —  
Part 2: Routine methods**

*Fruits, légumes et produits dérivés — Détermination de la teneur en acide ascorbique — Partie 2: Méthodes pratiques*

First edition — 1984-11-15

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ISO 6557-2:1984

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UDC 634.1/635.6 : 543.24 : 547.475.2

Ref. No. ISO 6557/2-1984 (E)

**Descriptors** : agricultural products, fruits and vegetable products, tests, determination of content, ascorbic acid.

## 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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6557/2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

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# Fruits, vegetables and derived products — Determination of ascorbic acid content — Part 2: Routine methods

## 1 Scope and field of application

This part of ISO 6557 specifies two routine methods for the determination of the ascorbic acid content<sup>1)</sup> of fruits, vegetables and derived products:

**method A:** 2,6-dichlorophenolindophenol titrimetric method;

**method B:** 2,6-dichlorophenolindophenol spectrometric method after extraction with xylene.

Method A can only be used in the absence of certain interferences (see 2.6).

Method B is applicable to derived fruit and vegetable products in strongly coloured solutions.

## 2 Method A: 2,6-dichlorophenolindophenol titrimetric method

### 2.1 Principle

Extraction of the ascorbic acid from a test portion using either oxalic acid solution or metaphosphoric acid-acetic acid solution. Titration with 2,6-dichlorophenolindophenol dyestuff until a salmon pink colour is obtained.

### 2.2 Reagents

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

#### 2.2.1 Extraction solution.

Use either a 2 % (*m/m*) oxalic acid solution or a metaphosphoric acid/acetic acid solution prepared as follows.

Dissolve 15 g of metaphosphoric acid in 40 ml of glacial acetic acid and 200 ml of water in a 500 ml one-mark volumetric flask, make up to the mark with water and filter immediately through filter paper into a glass bottle.

This solution can be kept for 7 to 10 days if stored in a refrigerator.

#### 2.2.2 2,6-dichlorophenolindophenol, dyestuff solution.

Dissolve 50 mg of the sodium salt of 2,6-dichlorophenolindophenol in 150 ml of hot (50 to 60 °C) water containing 42 mg of sodium hydrogen carbonate in a 200 ml one-mark volumetric flask, make up to the mark with water and filter. Store the solution in a dark brown bottle in a refrigerator.

As the dyestuff decomposes with time, fresh solution should be prepared periodically.

#### 2.2.3 Ascorbic acid, 1 g/l standard solution.

Weigh, to the nearest 0,01 mg, 50 mg of ascorbic acid which has been stored in a desiccator, transfer quantitatively to a 50 ml one-mark volumetric flask and make up to the mark with the extraction solution (2.2.1).

## 2.3 Apparatus

Usual laboratory equipment, and

### 2.3.1 Analytical balance.

### 2.3.2 Mixer.

### 2.3.3 Burette, of capacity 10 to 50 ml.

## 2.4 Procedure

### 2.4.1 Preparation of the test sample

If necessary, remove seeds and hard seed-cavity walls and then thoroughly mix the sample. Filter, and proceed with the determination on the filtrate.

Allow frozen or deep frozen products to thaw in a closed vessel and add the liquid formed during this process to the product before mixing.

### 2.4.2 Test portion

Weigh, to the nearest 0,1 mg, 10 to 100 g of the sample.

1) The ascorbic acid is determined as dehydroascorbic acid.

**2.4.3 Determination**

**2.4.3.1 Extraction**

Mix the test portion with the extraction solution (2.2.1) so that the volume, in millilitres, of the latter is numerically between 1 to 5 times the mass, in grams, of the test portion.

Filter the solution, discarding the first few millilitres of filtrate.

The ascorbic acid concentration in this test solution should be between 0,1 and 1 mg/ml.

**2.4.3.2 Standardization of the dyestuff solution**

Dilute a 5 ml aliquot portion of the standard ascorbic acid solution (2.2.3) with 5 ml of the extraction solution (2.2.1) and titrate rapidly with the dyestuff solution (2.2.2) until a salmon pink coloration, persisting for at least 5 s, is obtained. Repeat this procedure twice more, and record the volume of dyestuff solution used each time to the nearest 0,1 ml.

Proceed in the same way to establish the blank test, replacing the 5 ml of standard ascorbic acid solution with 5 ml of extraction solution.

Subtract the result of the blank test from the volumes of dyestuff solution used for the three standardization titrations and express the concentration of the dyestuff solution as the mass, in milligrams, of ascorbic acid equivalent to 1,0 ml of solution.

**2.4.3.3 Titration**

Take three aliquot portions of the filtrate obtained in 2.4.3.1 so that each contains about 2 mg of ascorbic acid, and titrate rapidly with the dyestuff solution until a salmon pink coloration, persisting for at least 5 s, is obtained. Take for the calculation (see 2.5) the arithmetic mean volume of dyestuff solution used.

**2.4.4 Blank test**

Carry out a blank test, proceeding as specified in 2.4.3, using the same volume of extraction solution as in 2.4.3.1, but omitting the test portion.

**2.4.5 Number of determinations**

Carry out three determinations on test portions taken from the same test sample.

**2.5 Expression of results**

The ascorbic acid content, expressed in milligrams per 100 g of product, is equal to

$$\frac{(V_0 - V_1) \times m_1}{m_0} \times 100$$

where

$m_0$  is the mass, in grams, of test portion in the aliquot portion taken for the titration;

$m_1$  is the mass, in milligrams, of ascorbic acid equivalent to 1,0 ml of the dyestuff solution (see 2.4.3.2);

$V_0$  is the volume, in millilitres, of dyestuff solution used for the titration;

$V_1$  is the volume, in millilitres, of dyestuff solution used in the blank test.

Take as the result the arithmetic means of the values obtained in the three determinations.

**2.6 Notes on procedure**

A number of interferences exist, in particular iron, copper, tin, reducing agents, hydrosulfides, sulfites and sulfur dioxide. Reducing agents, in particular, are present in products which have been overheated or stored for too long a period.

If the presence of any of these interferences is suspected, proceed as follows:

Add 2 drops of 0,05 % methylene blue solution to 10 ml of a solution containing equal volumes of the test solution and the extraction solution. Mix. The disappearance of the coloration within 5 to 10 s indicates the presence of interferences.

NOTE – Tin cannot be detected in this way, and the following procedure should be used.

Add 5 drops of 0,05 % carmine indigo to 10 ml of the test solution to which 10 ml of (1 + 3) hydrochloric acid has been added. Mix. The disappearance of the coloration within 5 to 10 s indicates the presence of tin or other interferences.

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**3 Method B: 2,6-dichlorophenolindophenol spectrometric method, after extraction with xylene**

**3.1 Principle**

Extraction of the ascorbic acid from a test portion using either oxalic acid solution or metaphosphoric acid-acetic acid solution. Quantitative reduction of 2,6-dichlorophenolindophenol dyestuff by the ascorbic acid, extraction of the excess dyestuff using xylene, and determination of the excess by spectrometric measurement at a wavelength of 500 nm.

**3.2 Reagents**

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

**3.2.1 Extraction solution.**

See 2.2.1.

**3.2.2 Sodium acetate/acetic acid, buffer solution, pH 4,0.**

Add 300 g of anhydrous sodium acetate to 700 ml of water and 1 000 ml of glacial acetic acid.

**3.2.3 2,6-dichlorophenolindophenol**, dyestuff solution.

See 2.2.2.

**3.2.4 Ascorbic acid**, 1 g/l standard solution.

See 2.2.3.

**3.2.5 Xylene**.

**WARNING** — In view of the narcotic properties of xylene in high concentrations, all operations involving its use should be carried out under a fume hood.

Check the purity of the xylene as follows:

Add ascorbic acid to a small quantity of the dyestuff solution (3.2.3) until the solution is decolorized, and shake with 10 ml of the xylene. Leave for 10 min. If there is any trace of colour in the xylene layer, the xylene shall be distilled.

The xylene used in the determination may be recovered by shaking with 20 % (*m/m*) sodium hydroxide solution to neutralize the acetic acid, followed by redistillation.

**3.3 Apparatus**

Usual laboratory equipment, and

**3.3.1 Analytical balance**.**3.3.2 Mixer**.**3.3.3 Microburettes**, of capacities 2, 5 and 10 ml.**3.3.4 Centrifuge tubes**, of capacity 25 ml, with glass stoppers.**3.3.5 Centrifuge**.**3.3.6 Spectrometer**, suitable for making measurements at a wavelength of 500 nm.**3.4 Procedure****3.4.1 Preparation of the test sample**

Proceed as specified in 2.4.1.

**3.4.2 Test portion**

Proceed as specified in 2.4.2.

**3.4.3 Determination****3.4.3.1 Extraction**

Proceed as specified in 2.4.3.1 in order to obtain a test solution containing between 0,05 and 0,5 mg of ascorbic acid per millilitre.

**3.4.3.2 Standardization of the dyestuff solution**.

Proceed as specified in 2.4.3.2.

**3.4.3.3 Reduction**

Transfer, by means of a pipette, 1 to 5 ml of the test solution to a centrifuge tube (3.3.4) and add an equal volume of the buffer solution (3.2.2). Immediately add an excess of the dyestuff solution (3.2.3), mix, and add 10 ml of the xylene (3.2.5). Stopper the tube and shake vigorously for 6 to 10 s. Centrifuge to separate the layers. Carefully remove the upper xylene layer and fill a spectrometric cell.

**3.4.3.4 Spectrometric measurement**

Measure the absorbance of the xylene layer at 500 nm.

**3.4.4 Blank test**

Measure the absorbance of the xylene (3.2.5) at 500 nm.

**3.4.5 Preparation of the calibration graph**

Transfer to each of four centrifuge tubes (3.3.4) the same volume of extraction solution as used for the determination (3.4.3.3). Add to each an equal volume of the buffer solution (3.2.2) and then add, respectively, 0,2; 0,4; 0,6; and 0,8 ml of the dyestuff solution (3.2.3).

Proceed as specified in 3.4.3.3.

Plot a graph of absorbance as a function of the volume of dyestuff solution added.

**3.4.6 Number of determinations**

Carry out two determinations on the same test sample.

**3.5 Expression of results**

The ascorbic acid content, expressed in milligrams per 100 g of product, is equal to

$$\frac{(V_0 - V_1) \times m_1}{m_0} \times 100$$

where

$m_0$  is the mass, in grams, of test portion in the aliquot portion taken for the determination;

$m_1$  is the mass, in milligrams, of ascorbic acid equivalent to 1,0 ml of the dyestuff solution;

$V_0$  is the volume, in millilitres, of dyestuff solution added in 3.4.3.3;

$V_1$  is the volume, in millilitres, of the excess of dyestuff corresponding to the absorbance measured in 3.4.3.4, read from the calibration graph.

### 3.6 Repeatability

The difference between the results of the two determinations (3.4.6), carried out simultaneously or in rapid succession by the same analyst on the same test sample, shall not exceed 3 % of the mean.

### 3.7 Notes on procedure

**3.7.1** If the product contains xylene-extractable pigments, carry out a hydroquinone blank correction as follows.

After measuring the absorbance of the xylene layer (see 3.4.3.4), add 2 drops of a semi-saturated solution of hydroquinone (prepared by adding twice the volume of acetone required to obtain a saturated solution of hydroquinone), mix, leave for 30 s and measure the absorbance again. Deduct this absorbance from the initial absorbance of the xylene layer.

**3.7.2** If the product has been overheated or stored for too long a period, or, in some cases, in the case of natural products (for example blackcurrant juice), a treatment with formaldehyde may be used to correct for the presence of reducing

substances not related to ascorbic acid. For this purpose, carry out a check test (control) in parallel with the determination, proceeding as specified in 3.4 up to the addition of the dyestuff. Before this addition, add to the test solution 1 ml of water and to the control solution 1 ml of 40 % formaldehyde solution. Leave for 10 min and then proceed with the determination.

From the calibration graph, determine the volume of dyestuff solution decolorized by the interferences and correct the result accordingly.

## 4 Test report

The test report shall show the method used and the result obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

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

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