



SLOVENSKI STANDARD

SIST ISO 6558-2:1995

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Fruits, vegetables and derived products -- Determination of carotene content -- Part 2:
Routine methods

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Fruits, légumes et produits dérivés -- Détermination de la teneur en carotènes -- Partie 2:
Méthodes pratiques

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Ta slovenski standard je istoveten z:

ISO 6558-2:1992

ICS:

67.080.01	Sadje, zelenjava in njuni proizvodi na splošno	Fruits, vegetables and derived products in general
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INTERNATIONAL STANDARD

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Fruits, vegetables and derived products — Determination of carotene content —

Part 2:

Routine methods

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

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Partie 2: Méthodes pratiques



Reference number
ISO 6558-2:1992(E)

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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.

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.

International Standard ISO 6558-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 3, *Fruit and vegetable products*.

[SIST ISO 6558-2:1995](https://standards.iteh.ai/catalog/standards/sist/8aad6fd5-721e-49d3-a5bb-b8eb925f1e9/sist-iso-6558-2-1995)

ISO 6558 consists of the following parts, under the general title *Fruits, vegetables and derived products — Determination of carotene content*:

- *Part 1: Reference method*
- *Part 2: Routine methods*

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Fruits, vegetables and derived products — Determination of carotene content —

Part 2: Routine methods

1 Scope

This part of ISO 6558 specifies two routine methods for the determination of the carotene ($C_{40}H_{56}$) content present in the edible parts of, or added to, fruits, vegetables and derived products.

Method A is for the determination of the carotene content in products having a fat content of less than or equal to 5 % (m/m)¹⁾.

Method B is for the determination of the carotene content in products having a fat content of more than 5 % (m/m)¹⁾.

2 Method A: Determination of carotene content in products having a fat content of less or equal to 5 % (m/m)

2.1 Principle

Extraction of carotenes using a mixture of petroleum ether and acetone. Elimination of the acetone, separation of the carotenes from the carotenoids by column chromatography and determination of the carotene content by spectrometry.

2.2 Reagents and materials

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

2.2.1 Petroleum ether, boiling range 50 °C to 70 °C.

2.2.2 Extraction mixture, prepared as follows.

Dissolve 0,2 g of hydroquinone in 40 ml of acetone and then add 160 ml of the petroleum ether (2.2.1).

2.2.3 Sodium sulfate, anhydrous.

Dry at 100 °C for 2 h to 3 h and keep in a hermetically closed container.

2.2.4 Quartz sand, acid washed and dried in a muffle furnace.

2.2.5 Column packing, use one of the column packings specified in 2.2.5.1 to 2.2.5.4.

2.2.5.1 Aluminium oxide, deactivated by suspension in water, prepared as follows.

Heat the aluminium oxide for 3 h in a muffle furnace at 500 °C, cool and keep in a desiccator. Before use, transfer 50 g of the aluminium oxide thus prepared into a flask with a ground glass joint, add 5,5 ml of water and mix thoroughly until a fine uniform suspension is obtained. The pH of the suspension thus obtained is between 9 and 10. The suspension may be kept in a closed flask for 24 h.

2.2.5.2 Magnesium oxide/powdered glass mixture, 1 part to 2 parts by mass.

2.2.5.3 Aluminium oxide/anhydrous sodium sulfate mixture, 3 parts to 1 part by mass.

2.2.5.4 Aluminium oxide/calcium hydroxide mixture, 1 part to 1 part by mass.

2.2.6 Sodium chloride, 300 g/l solution.

1) The limit of 5 % (m/m) adopted for the fat content is arbitrary.

2.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

2.3.1 Spectrometer, suitable for carrying out measurements at 450 nm, equipped with cells of 10 mm path length.

2.3.2 Witt filter apparatus, with ground-in funnel, ground glass cover and side tube, for filtering into a beaker or other vessel under diminished pressure.

2.3.3 Water-pump.

2.3.4 Water-bath, capable of being maintained at a temperature of 30 °C to 35 °C.

2.3.5 Vacuum evaporator.

2.3.6 Chromatography column, having a diameter between 10 mm and 15 mm.

2.3.7 Sintered (fritted) filter, grade P40.

2.3.8 Cotton wool, soaked with petroleum ether.

2.3.9 Laboratory mortar and pestle.

2.3.10 Desiccator, provided with an efficient desiccant.

2.3.11 Conical flask, of 500 ml capacity.

2.3.12 Round-bottomed flasks, of 100 ml and 500 ml capacity, with ground glass joints.

2.3.13 Separating funnel, of 1 000 ml capacity.

2.3.14 One-mark volumetric flasks, of 50 ml and 100 ml capacity.

2.4 Preparation of the test sample

2.4.1 Liquid non-homogeneous products (e.g. juices, concentrates and syrups)

Mix the laboratory sample thoroughly.

2.4.2 Viscous products (e.g. marmalade and fruits in syrup) and **solid products** (e.g. fruits and vegetables)

Remove seeds and hard seed-cavity walls, if necessary, and then mix the laboratory sample thoroughly.

Weigh all seeds etc. removed from the product to allow for correction of the analytical result obtained for the residue.

2.4.3 Frozen and deep-frozen products

Thaw the laboratory sample in a closed container. If necessary, perform step 2.4.2. Add the liquid formed during thawing and mix the product thoroughly.

2.5 Procedure

IMPORTANT — Carry out the analysis in a dark place away from direct light.

2.5.1 Test portion

Weigh a quantity (generally 1 g to 10 g) of the prepared test sample (2.4) containing between 5 µg and 150 µg of carotene.

2.5.2 Preparation of the test solution

2.5.2.1 Transfer quantitatively the test portion to a mortar (2.3.9) and add about 20 ml of the extraction mixture (2.2.2). Add 20 g of the anhydrous sodium sulfate (2.2.3) and 30 g of quartz sand (2.2.4) and grind well. Decant the extract obtained through a filter (2.3.7) into a 500 ml conical flask (2.3.11). Repeat the extraction until a colourless extract is obtained, collecting the filtered extracts in the conical flask. Transfer quantitatively the residue to the filter and wash it with the extraction mixture, collecting the filtrate in the conical flask.

2.5.2.2 Transfer the combined extracts into a 1 000 ml separating funnel (2.3.13) and wash several times with a total of 300 ml to 400 ml of water in order to eliminate the acetone. Agitate the extract carefully in order to avoid the formation of an emulsion. If an emulsion is formed, repeat the extraction using another test portion and carry out the washing procedure as described, but using a 300 g/l sodium chloride solution (2.2.6) in place of water.

2.5.2.3 Add to the washed extract 15 g of the anhydrous sodium sulfate (2.2.3), mix and leave to stand for 15 min. Transfer the solution through a filter (2.3.7), half-filled with anhydrous sodium sulfate, into a 500 ml round-bottomed flask (2.3.12). Wash the sodium sulfate with three 10 ml quantities of petroleum ether (2.2.1), collecting the washings in the filtrate.

2.5.2.4 Concentrate the combined filtrate in a vacuum evaporator (2.3.5) over a water-bath (2.3.4) set at 30 °C to 35 °C, transfer it quantitatively into a 100 ml (or 50 ml) one-mark volumetric flask (2.3.14) and make up to the mark with petroleum ether (V_1).

NOTE 1 If an emulsion forms, 10 ml of ethanol may be added.

2.5.3 Elution

IMPORTANT — Throughout the elution procedure the column shall always be filled with petroleum ether to a level above the surface of the adsorbent.

2.5.3.1 Place a degreased cotton plug (2.3.8) in the lower, narrow, part of the chromatography column (2.3.6). Place the column on the Witt apparatus (2.3.2) and, while shaking gently, introduce in a uniform way the deactivated aluminium oxide suspension (2.2.5.1) (but see below), or the magnesium oxide/powdered glass mixture (2.2.5.2) or the aluminium oxide/anhydrous sodium sulfate mixture (2.2.5.3) to a height of 15 cm to 20 cm. Aluminium oxide is not a suitable adsorbent for the separation of lycopene carotenoids. When analysing products containing lycopene (such as tomatoes and tomato products), use an aluminium oxide/calcium hydroxide mixture (2.2.5.4) as adsorbent.

Use as a collecting container a 100 ml round-bottomed flask (2.3.12). Connect the Witt apparatus to a water pump (2.3.3).

2.5.3.2 Fill the column with petroleum ether. When the level of the petroleum ether falls to about 1 cm above the surface of the adsorbent, introduce about 5 ml to 20 ml (accurately measured) of the test solution (V_2), depending on the intensity of its colour. When the level of the test solution is about 1 cm above the surface of the adsorbent, add 30 ml of petroleum ether.

NOTE 2 During the washing procedure, α -, β - and γ -carotenes move down the column as indicated by a yellowish zone, and are eluted quantitatively, while the carotenoids (xanthophyll, cryptoxanthine, etc.) are retained in the upper layer of the adsorbent.

2.5.3.3 Proceed with the elution until the eluate is colourless (normally after about 20 ml of petroleum ether has been run off).

2.5.3.4 Depending on the colour intensity of the eluate, adjust its volume (by concentration or dilution with petroleum ether, as necessary) such that 1 ml of eluate contains 0,4 μg to 3,0 μg of carotene. Note the final volume of eluate (V_3).

2.5.4 Spectrometric measurements

Carry out the spectrometric measurements of the eluate against a petroleum ether blank at a wavelength of 450 nm, using cells of 10 mm path length.

The molar absorption coefficient $A_{1\text{ cm}}^{1\%}$ of a β -carotene reference solution in petroleum ether at 450 nm is 2 530.

2.6 Expression of results

Calculate the carotene content, expressed as β -carotene, using the formula

$$w(\text{C}_{40}\text{H}_{56}) = \frac{AV_1V_3}{0,25V_2m}$$

where

$w(\text{C}_{40}\text{H}_{56})$ is the carotene content, in micrograms per gram of product;

A is the absorbance of the eluate;

V_1 is the volume, in millilitres, of the test solution (i.e. 100 ml or 50 ml);

V_2 is the volume, in millilitres, of the test solution used for chromatography;

V_3 is the final volume, in millilitres, of the eluate;

0,25 is the correction factor for β -carotene;

m is the mass, in grams, of the test portion.

3 Method B: Determination of carotene content in products having fat contents of more than 5 % (m/m)

3.1 Principle

Saponification of a test portion by treatment with an alcoholic potassium hydroxide solution. Extraction of carotenoids using a mixture of petroleum ether and diethyl ether, and determination of the carotene content by spectrometry.

3.2 Reagents and materials

Use the reagents and materials specified in 2.2 except for 2.2.2, 2.2.4 and 2.2.6 and, in addition, the following, also of recognized analytical grade.

3.2.1 Nitrogen.

3.2.2 Ethanol, 96 % (V/V) solution.

3.2.3 Potassium hydroxide, 500 g/l solution.

In a graduated cylinder provided with a ground glass stopper, dissolve 5,0 g of potassium hydroxide in distilled water, and make up to 10 ml with water.

Prepare the solution immediately before use.

3.2.4 Pyrogallol, basic solution, prepared as follows.

Prepare a 250 g/l pyrogallol solution by dissolving,

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in a 25 ml one-mark volumetric flask, 6,25 g of pyrogallol in water. Make up to the mark with water.

Prepare a 600 g/l potassium hydroxide solution by dissolving, in a 100 ml one-mark volumetric flask, 60 g of potassium hydroxide in water. Make up to the mark with water.

Prepare the basic solution by mixing 15 ml of the pyrogallol solution with 90 ml of the potassium hydroxide solution.

3.2.5 Extraction mixture, prepared as follows.

Mix 1 part of petroleum ether with 1 part of diethyl ether, free from peroxides.

3.2.6 Potassium hydroxide, 30 g/l solution.

In a 50 ml one-mark volumetric flask, dissolve 1,5 g of potassium hydroxide in water, and make up to the mark with water.

3.2.7 Phenolphthalein, 1 % solution.

Dissolve 1,0 g of phenolphthalein in 60 % (V/V) ethanol solution and make up to 100 ml with the same ethanol.

3.2.8 Antioxidant, e.g. pyrogallol, hydroquinone, ascorbic acid.

3.3 Apparatus

The apparatus specified in 2.3 except for 2.3.9, 2.3.11 and 2.3.13 and, in addition, the following.

3.3.1 Apparatus for saponification, under reflux in an inert gas flow, incorporating an absorption bottle containing pyrogallol basic solution (3.2.4) for eliminating traces of oxygen from the gas.

3.3.2 Separating funnel, of 250 ml capacity.

3.4 Preparation of the test sample

Prepare the test sample in accordance with 2.4.

3.5 Procedure

3.5.1 Test portion

Weigh into a 100 ml round-bottomed flask (2.3.12) a quantity of the prepared test sample (3.4) containing between 5 µg and 150 µg of carotene.

3.5.2 Preparation of the test solution

3.5.2.1 Add to the test portion 30 ml of the ethanol solution (3.2.2), 3 ml of the potassium hydroxide solution (3.2.3) and some antioxidant (3.2.8) and connect the flask to the saponification apparatus (3.3.1). Start up the flow of nitrogen (3.2.1). Carry out the saponification under reflux, keeping the solution boiling by means of a water-bath, for 30 min.

3.5.2.2 Cool the mixture and transfer it quantitatively, rinsing with about 20 ml of distilled water, into a 250 ml separating funnel (3.3.2). Extract the non-saponifiable substances by stirring gently twice with 50 ml and twice with 20 ml of the extraction mixture (3.2.5). Wash carefully the obtained extract with 50 ml of the potassium hydroxide solution (3.2.6) and then with water until the extract is completely free from alkali (phenolphthalein test; see 3.2.7).

3.5.2.3 Remove water from the extract in accordance with the procedure specified in 2.5.2.3.

3.5.2.4 Concentrate the extract in accordance with the procedure specified in 2.5.2.4.

3.5.3 Elution and spectrometric measurements

Carry out the elution and spectrometric measurement in accordance with the procedures specified in 2.5.3 and 2.5.4.

3.6 Expression of results

Calculate the carotene content, expressed as β -carotene, using the formula given in 2.6.

4 Repeatability

The absolute difference between two independent single test results, obtained using 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, should not be greater than 5 % of the arithmetic mean of the two results.

5 Test report

The test report shall specify the method used (i.e. ISO 6558-2, method A or B) and the results obtained. It shall also mention all operating details not specified in this part of ISO 6558, or regarded as optional, together with details of any incidents which may have influenced the results.

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