# SLOVENSKI STANDARD
# SIST EN 12136:1998

**01-junij-1998**

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**Sadni in zelenjavni sokovi - Določevanje celotnih karotenoidov in posameznih frakcij karatenoidov**

Fruit and vegetable juices - Determination of total carotenoid content and individual carotenoid fractions

Frucht- und Gemüsesäfte - Bestimmung des Gesamtcarotinoidgehaltes sowie einzelner Carotinoid-Fraktionen



Jus de fruits et de légumes - Détermination de la teneur en caroténoides totaux et en fractions individuelles de caroténoides



**Ta slovenski standard je istoveten z:** **EN 12136:1997**

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|---|---|---|
| 67.160.20 | Brezalkoholne pijače | Non-alcoholic beverages |

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English version

# Fruit and vegetable juices - Determination of total carotenoid content and individual carotenoid fractions

Jus de fruits et de légumes - Détermination de la teneur en caroténoïdes totaux et en fractions individuelles de caroténoïdes

Frucht- und Gemüsesäfte - Bestimmung des Gesamtcarotinoidgehaltes sowie einzelner Carotinoid-Fraktionen

This European Standard was approved by CEN on 6 September 1997.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.



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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

**Central Secretariat: rue de Stassart, 36 B-1050 Brussels**

Contents

## Foreword

This European Standard has been prepared by Technical Committee CEN/TC 174 "Fruit and vegetable juices - Methods of analysis", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 1998, and conflicting national standards shall be withdrawn at the latest by March 1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

## 1 Scope

This European Standard specifies a method for the determination of total carotenoid content and individual carotenoid fractions of fruit and vegetable juices and related products.

## 2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

| | | |
|---|---|---|
| EN ISO 3696 | 1995 | Water for analytical laboratory use - Specification and test methods (ISO 3696:1987) |
| ISO 5725 | 1986 | Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests. |

## 3 Definitions and symbols



### 3.1 Definitions

For the purposes of this standard, the following definitions apply :



#### 3.1.1 Carotenoid fraction I (mainly hydrocarbons)

Material eluted with petroleum ether, as described in 7.3.2.

#### 3.1.2 Carotenoid fraction II (crypotxanthin esters)

Material eluted with elution mixture A (5.7) as described in 7.3.2.

#### 3.1.3 Carotenoid fraction III (xantophyll esters)

Material eluted with elution mixture B (5.8), as described in 7.3.2.

#### 3.1.4 Carotenoid fraction IV

Material eluted with acetone (5.3) as described in 7.3.2.

### 3.2 Symbols

For the purposes of this standard, the following symbol apply :

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|---|---|
| *g* | Acceleration due to gravity at the surface of the earth (9,81 m/s²). |

## 4 Principle

Quantitative adsorptive precipitation of carotenoids by zinc hexacyanoferrate (II) by treatment with Carrez I and Carrez II solutions. Extraction of carotenoids from precipitate using acetone and transfer of the solution to petroleum ether. Determination of the total carotenoid content by spectrometry. Fractionation of the carotenoids using chromatography column on aluminium oxide. Individual determination of the single carotenoid fractions spectrometrically. Calculation of the total carotenoid content and the single fractions as β-carotene, expression of content of the individual carotenoid fractions as a percentage of total carotenoid content.

## 5 Reagents

Use only reagents of recognized analytical grade and only water in accordance with at least grade 3 of EN ISO 3696:1995.

### 5.1 Carrez I solution

Dissolve 15,0 g of potassium hexacyanoferrate (II) trihydrate ($K_4Fe(CN)_6 . 3H_2O$) in water and dilute to 100 ml with water.

### 5.2 Carrez II solution



Dissolve 30 g of zinc sulfate heptahydrate ($ZnSO_4 . 7H_2O$) in water and dilute to 100 ml with water.



### 5.3 Acetone ($CH_3COCH_3$)

### 5.4 Petroleum ether, boiling range 40 °C to 60 °C

Clean the petroleum ether on a column filled with aluminium oxide ($Al_2O_3$) (5.9).

### 5.5 Sodium sulfate ($Na_2SO_4$), anhydrous

### 5.6 Toluene ($C_6H_5CH_3$)

### 5.7 Elution mixture A

Mix four volums of petroleum ether (5.4) with one volum of toluene (5.6).

### 5.8 Elution mixture B

Mix two volums of petroleum ether (5.4) with one volum of toluene (5.6).

**5.9 Aluminium oxide** ($Al_2O_3$) activated, neutral, acitivity grade I, for column chromatography and adsorptive filtration

**5.10 Aluminium oxide** ($Al_2O_3$), deactivated partly by suspension in water

Transfer 100 g of aluminium oxide (5.9) into a flask with a ground glass joint, add 12 ml of water and mix thoroughly until a fine uniform suspension is obtained. Keep the flask well stoppered. The uniform suspension obtained should stand for 2 h.

NOTE: The deactivated aluminium oxide represents the activity level IV-V. Since the activity changes after long periods of storage, the required quantities should be used between 2 h and 24 h after preparation.

## 6 Apparatus

**WARNING : Since the determination involves the use of volatile flammable solvents, electrical apparatus employed has to be in accordance with legislation relating to the hazards in using such solvents.**

Usual laboratory apparatus and, in particular, the following



**6.1 Spectrometer** capable for measuring at a wavelength of 450 nm



**6.2 Glass or quartz cuvettes** of 10 mm optical path length, and which have no significant absorption at a wavelength of 450 nm



**6.3 Centrifuge,** capable of producing a centrifugal acceleration of 2 000 *g* at the base of the centrifuge tube (6.4).

NOTE: The rotational frequency required to give correct centrifugal acceleration can be calculated from the following equation :

$$a = 11{,}18 \cdot r \cdot (n/1\,000)^2 \qquad (1)$$

where :

*a* is the centrifugal acceleration ;

*r* is the radius of the centrifuge in centimetres, measured from the mid point (the centrifuge axis) to the bottom of the centrifuge tube when swung out ;

*n* is the rotation frequency per minute.

**6.4 Centrifuge tubes** of 60 ml to 100 ml capacity

**6.5 Chromatography tube** having a length of between 250 mm and 300 mm and an inner diameter of 20 mm equipped with a polytetrafluorethylene (PTFE) stopcock

**6.6 Rotary vacuum evaporator**

**6.7 Separating funnel** of 200 ml capacity

**6.8 Volumetric flask** of 100 ml capacity

**6.9 Round bottom flask** of 250 ml capacity

**6.10 Graduated flasks** of suitable capacity

## 7 Procedure

Carotenoids are sensitive to light and heat. Carry out the analysis in a place protected from direct sunlight or UV exposure.

### 7.1 Preparation of the test sample

Normally products shall not be pre-treated, however dilution may be necessary and their analysis by this method shall be on a volumetric basis, results being expressed per litre of sample. The analysis of concentrated products may also be carried out on a volumetric basis, after dilution to a known relative density. In this case, the relative density shall be indicated. Based on a weighed sample and taking the dilution factor for analysis into account, the results may also be expressed per kilogram of product. In products with a high viscosity and/or a very high content of cells (for example pulp), a determination on the basis of a weighed test sample is the usual procedure.



### 7.2 Determination of total carotenoid content

Shake the liquid test sample thoroughly and pipette a test portion of 5 ml to 50 ml ($V_1$) into a centrifuge tube (6.4). Dilute to 50 ml with water if necessary and add 1 ml each of Carrez I (5.1) and Carrez II (5.2) solutions. Mix thoroughly, allow to stand for about 2 min and then centrifuge at 2 000 *g* (6.3) for about 5 min.

> NOTE 1 : The absorbance of the final extract made up to 100 ml ($V_2$) should not exceed 0,5 units. To allow for this, it is necessary to adjust the amount of sample taken ($V_1$) for the analysis. The following values are given as guidance : 25 ml for orange juice, 50 ml for orange nectar, 5 ml to 20 ml for passion fruit juice, 25 ml for passion fruit nectar, and 20 ml for tangerine juice.

Remove the nearly colourless supernatant solution by decantation. Add 40 ml of acetone (5.3) to the precipitate in the centrifuge tube, mix thoroughly with a glass rod and continue to stir for about 3 min. Centrifuge again for about 5 min and decant the supernatant yellow acetone solution into a separating funnel (6.7). Add 50 ml of petroleum ether (5.4) using a measuring cylinder (6.10).