SLOVENSKI STANDARD
SIST EN 12630:1999

01-maj-1999

Sadni in zelenjavni sokovi - Določanje vsebnosti glukoze, fruktoze, sorbitola in saharoze - Metoda uporabe tekočinske kromatografije z visoko ločljivostjo

Fruit and vegetable juices - Determination of glucose, fructose, sorbitol and sucrose contents - Method using high performance liquid chromatography

Frucht- und Gemüsesäfte - Bestimmung des Glucose-, Fructose-, Sorbit- und Saccharosegehaltes - Hochleistungs-flüssigchromatographisches Verfahren

Jus de fruits et de légumes - Dosage du glucose, du fructose, du sorbitol et du saccharose - Méthode par chromatographie liquide haute performance

Ta slovenski standard je istoveten z: **EN 12630:1999**

ICS:

67.160.20 Brezalkoholne pijače Non-alcoholic beverages

SIST EN 12630:1999 **en**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 12630

February 1999

ICS 67.160.20

Descriptors: fruit-and vegetable juices, chemical analysis, determination of content, glucose, fructose, sorbitol, chromatographic analysis, high performance liquid chromatography

English version

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This European Standard was approved by CEN on 8 January 1999.

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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of an identical text or by endorsement, at the latest by August 1999, and conflicting national standards shall be withdrawn at the latest by August 1999.

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 high performance liquid chromatographic method for the determination of the glucose, fructose, sorbitol and sucrose contents in fruit and vegetable juices and related products.

This method does not allow the determination of sucrose in the presence of maltose due to overlapping of the peaks.

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)

3 Symbols and abbreviations

3.1 Symbols

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

- c substance concentration ;
- ρ mass concentration ;
- g acceleration due to gravity at the surface of the earth (9,81 ms^{-2}).

3.2 Abbreviations

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

- HPLC High performance liquid chromatography ;
- EDTA Ethylenediaminetetraacetic acid.

4 Principle

The sugars and sorbitol are separated on a cation-exchange resin (sulfonated polystyrene-divinylbenzene copolymer in the Ca^{2+} form) by isocratic elution using an aqueous solution of calcium disodium-EDTA as the mobile phase. The sugars and sorbitol are detected using a differential refractive index detector and quantified using the external standard method.

5 Reagents

5.1 General

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

5.2 Calcium disodium-EDTA solution, $c(C_{10}H_{12}N_2O_8CaNa_2.xH_2O) = 0,1$ mmol/l

In one litre of HPLC grade water (5.1).

5.3 Standard solution

Prepare a standard solution of glucose, fructose, sorbitol and sucrose at a concentration of 10 g/l in water (5.1).

6 Apparatus

Usual laboratory apparatus and, in particular, the following :

6.1 HPLC-equipment : consisting of HPLC pump, HPLC column (6.2) and differential refractive index detector, column heater.

6.2 HPLC-column : polystyrene-divinylbenzene sulfonated cation exchange column in the calcium form, particle size 10 µm, length 30 cm, internal diameter 6,5 mm, a column with other dimensions can be used if it gives similar results.

6.3 Syringe filter : hydrophillic syringe filter, non-sterile, with 0,45 µm pore size.

6.4 Centrifuge : capable of producing a centrifugal acceleration of 1 400 *g*.

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

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

where :

a is the centrifugal acceleration ;

r is the radius of the centrifuge in centimetres, measured from the midpoint (the centrifuge axis) to the bottom of the centrifuge tube when swung outwards ;

n is the rotational frequency per minute.

6.5 Centrifuge tubes

7 Procedure

7.1 General

Analysis by this method shall be on a volumetric basis, the 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), determination on the basis of a weighed test sample is the usual procedure.

7.2 Preparation of test sample

Mix cloudy samples well before dilution. The single strength juice shall be diluted one volume part plus four volume parts of water and centrifuged at 1 400 *g* for 15 min. The sample is then filtered through the syringe filter (6.3) and is ready for HPLC analysis.

7.3 HPLC conditions

Solvent : Calcium disodium-EDTA solution (5.2)

Flow rate : 0,5 ml/min

Column temperature : 90 °C (or as defined in the column manufacture's instructions)

Injection volume : 10 µl (typically).

NOTE : The differential refractive index detector should be held at a constant temperature around ambient (25 °C to 35 °C).

7.4 HPLC analysis

After equilibration of the HPLC system and the column, determine the retention times of the sugars and sorbitol by injection of solutions of the single compounds. This stage shall also be used to confirm that no inversion of sucrose occurs on the column under the conditions chosen. Subsequent analysis can be carried out using a mixed standard. The standard solutions are injected at regular intervals between test samples (for example typically every fifth or seventh injection).

A typical chromatogram for a mixed standard of the sugars and sorbitol is given in Annex C.

8 Calculation

The sugars and sorbitol concentrations are determined using the external standard method, by peak areas or peak heights. During the calculation allow for any dilution factor and the relationship of the value to mass or volume. If a concentrated product has been diluted to single strength, report the relative density of the single strength sample. Calculate the concentrations of the sugars and sorbitol ρ using the following equation :

$$\rho = \frac{P}{RF} \times F \qquad (2)$$

where :

P is the peak area or peak height of the sugar or sorbitol under investigation depending on which method is chosen ;

F is the dilution factor (5 for juices, for diluted concentrates the factor shall be calculated) ;

RF is the appropriate response factor for the particular sugar or sorbitol and is calculated using the following equation :

$$RF = \frac{P_s}{\rho_s} \qquad (3)$$

where :

P_s is the peak area or peak height of the particular sugar or sorbitol in the standard chromatogram ;

ρ_s is the mass concentration of the particular sugar or sorbitol in the standard solution.

Report the concentrations of glucose, fructose, sorbitol and sucrose in grams per litre to one decimal place.

NOTE 1 : Separate response factor should be determined for glucose, fructose, sorbitol and sucrose using the equation above and the appropriate data.

NOTE 2 : When this method was assessed in a collaborative study the concentrations of the sugars were also assessed using enzymatic procedures. It was found that the enzymatic methods gave a better reproducibility for glucose and fructose whereas the HPLC method gave a better reproducibility for sucrose.

9 Precision

Details of the interlaboratory test on precision of the method are summarised in annex B. The values derived from the interlaboratory test may not be applicable to analyse concentration ranges and matrices other than given in annex B.