SLOVENSKI STANDARD
SIST EN 12146:1998

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Sadni in zelenjavni sokovi - Encimatsko določevanje saharoze - NADP spektrometrijska metoda

Fruit and vegetable juices - Enzymatic determination of sucrose content - NADP spectrometric method

Frucht- und Gemüsesäfte - Enzymatische Bestimmung des Saccharosegehaltes - Spektralphotometrisches Verfahren mit NADP

Jus de fruits et de légumes - Dosage enzymatique du saccharose - Méthode spectrométrique par le NADP

Ta slovenski standard je istoveten z: **EN 12146:1996**

ICS:

67.160.20	Brezalkoholne pijače	Non-alcoholic beverages

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English version

Fruit and vegetable juices - Enzymatic determination of sucrose content - NADP spectrometric method

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Frucht- und Gemüsesäfte - Enzymatische Bestimmung des Saccharosegehaltes - Spektralphotometrisches Verfarhen mit NADP

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Contents

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 174 "Fruit and vegetable juices - Method 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of an identical text or by endorsement, at the latest by March 1997, and conflicting national standards shall be withdrawn at the latest by March 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard : Austria, Belgium, 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 an enzymatic method for the determination of the content of sucrose in 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 5725:1986	Precision of test methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory tests.

3 Symbols and abbreviations

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

ATP	Adenosine-5'-triphosphate;
ADP	Adenosine-5'-diphosphate;
NADP	ß-Nicotinamide-adenine-dinucleotidephosphate
NADPH	ß-Nicotinamide-adenine-dinucleotidephosphate (reduced form);
G-6-P	Glucose-6-phosphate;
HK	Hexokinase (EC 2.7.1.1) [1];
G6P-DH	Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [1];
BF	ß-Fructosidase (EC 3.2.1.26) [1];
IU	One International Unit of enzyme activity, which catalyses the conversion of 1 µmol of substrate per min at 25 °C;
c	Substance concentration;
ρ	Mass concentration.

[1] Enzyme Commission (EC) ; Classification System
Enzyme Handbook, Springer, Berlin 1969

4 Principle and reactions

4.1 Principle

Sucrose is enzymatically hydrolysed (inverted) by the action of BF in a diluted test sample to give equal amounts of D-glucose and D-fructose. The D-glucose formed is then phosphorylated in the C-6 position in an enzyme-catalysed reaction involving ATP and HK.

In a concomitant reaction, G-6-P is converted stoichiometrically to 6-phosphogluconate in the presence of NADH, the reaction being catalysed by the enzyme G6P-DH and an amount of NADPH equivalent to the amount of D-glucose present in the test sample being formed (4.2)

The quantification of the NADPH formed and hence the content of D-glucose and sucrose is performed by spectrometry.

In juices where there is a low level of sucrose (less than 5 g/l) and a higher level of glucose it is impossible to obtain an accurate quantification of sucrose via the conventional enzymatic determination. In these circumstances the glucose should be removed, by reaction with iodine at an alkaline pH, prior to quantification of the sucrose.

4.2 Reactions

$$\text{sucrose} + H_2O \xrightarrow{BF} \text{D-glucose} + \text{D-fructose} \quad (1)$$

$$\text{glucose} + \text{ATP} \xrightarrow{HK} \text{G-6-P} + \text{ADP} \quad (2)$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{G6P\text{-}DH} \text{6-phosphogluconate} + \text{NADPH} + H^+ \quad (3)$$

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.

5.2 Sodium hydroxide solutions

Sodium hydroxide solutions are prepared at c (NaOH) = 5 mol/l, c (NaOH) = 4 mol/l and c (NaOH) = 2 mol/l

5.3 Citrate buffer pH = 4,6

Dissolve 6,9 g of citric acid monohydrate ($C_6H_8O_7 . H_2O$) and 9,1 g of trisodium citrate dihydrate ($C_6H_5Na_3O_7 . 2H_2O$) in 150 ml of water, adjust to pH = 4,6 with sodium hydroxide solution (c (NaOH) = 2,0 mol/l) (5.2) and dilute to 200 ml with water. The buffer is stable for at least 1 year at 4 °C.

5.4 BF solution

Dissolve 10 mg of ß-fructosidase, ρ = 5 mg/ml, about 750 IU/ml, in 2 ml of the citrate buffer (5.3). The solution is stable for at least 1 week at 4 °C.

5.5 Triethanolamine buffer pH = 7,6

Dissolve 14,0 g of triethanolamine hydrochloride and 0,25 g of magnesium sulfate heptahydrate ($MgSO_4 . 7H_2O$) in 80 ml of water, adjust to pH = 7,6 with approximately 5 ml of sodium hydroxide solution (c (NaOH) = 5 mol/l) and dilute to 100 ml with water. The buffer is stable for at least 4 weeks at 4 °C.

5.6 NADP solution

Dissolve 60 mg of ß-nicotinamide-adenine-dinucleotide phosphate-disodium salt (ß-NADP-Na_2) in 6 ml of water. The solution is stable for at least 4 weeks at 4 °C.

5.7 ATP solution

Dissolve 300 mg of adenosine-5'-triphosphate-disodium salt (ATP-$Na_2H_2 . 3H_2O$) and 300 mg of sodium hydrogen carbonate ($NaHCO_3$) in 6 ml of water. The solution is stable for at least 4 weeks at 4 °C.

5.8 HK/G6P-DH enzyme suspension

Suspend hexokinase, ρ (HK) = 2 mg/ml, about 280 IU/ml (with D-glucose serving as the substrate in the presence of ATP) and G6P-DH, ρ (G6P-DH) = 1 mg/ml, about 140 IU/ml (with G-6-P as substrate) in ammonium sulfate solution, c $(NH_4)_2SO_4$) = 3,2 mol/l. The suspension is stable for at least 1 year at 4 °C.

5.9 Iodine solution

Dissolve 130 g of iodine and 150 g of potassium iodide in sufficient quantity of water in a 1 l volumetric flask, when dissolved dilute to volume with water.

5.10 Sulfuric acid

Prepare a solution of sulfuric acid at *c* (H_2S0_4) = 0,5 mol/l from an appropriate concentrated standard solution.

5.11 Sodium sulfite solutions

Prepare a saturated solution of sodium sulfite in water (solubility $\rho(Na_2SO_3) = 12{,}54\ g/100ml$ at 0 °C and 28,3 g/100 ml at 80 °C). From this concentrated solution prepare a dilute solution by carrying out a 1 to 10 dilution in water.

5.12 Phenolphthalein solution

Prepare a solution of phenolphthalein (ρ = 0,5 g/100 ml) in ethanol.

6 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.1 Enzyme test pipettes, graduated along the stem only, with long ungraduated delivery tip.

6.2 Pipettes, with accuracy equivalent to 6.1 (alternative to 6.1) e.g. positive displacement capillary pipettes.

6.3 Cuvettes, made of quartz, glass or plastics of 10 mm path length, and which do not have a significant absorption at the wavelengths of interest (e.g. 334 nm, 340 nm or 365 nm).

6.4 Spectral-line photometer, with mercury lamp and filters for measuring at wavelengths of 334 nm or 365 nm.

6.5 Spectrometer, (variable wavelength) for measuring at a wavelength of 340 nm (alternative to 6.4).

7 Procedure

7.1 Preparation of the test sample

7.1.1 Normal sample preparation

The fruit juice shall be diluted so that the sucrose/glucose concentration is between 0,1 g/l and 1,5 g/l. This solution shall be used directly and will normally not require any pre-treatment. The 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