SLOVENSKI STANDARD
SIST EN 1140:1996

01-avgust-1996

Sadni in zelenjavni sokovi - Encimsko ugotavljanje vsebnosti D-glukoze in D-fruktoze - NADH spektrometrijska metoda

Fruit and vegetable juices - Enzymatic determination of D-glucose and D-fructose content - NADPH spectrometric method

Frucht- und Gemüsesäfte - Enzymatische Bestimmung der Gehalte an D-Glucose und D -Fructose - Spektralphotometrische Bestimmung von NADPH

Jus de fruits et de légumes - Dosage enzymatique du glucose-D et du fructose-D - Méthode spectrométrique par le NADPH

Ta slovenski standard je istoveten z: **EN 1140:1994**

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English version

Fruit and vegetables juices - Enzymatic determination of D-glucose and D-fructose content - NADPH spectrometric method

Jus de fruits et de légumes - Dosage enzymatique du glucose-D et du fructose-D - Méthode spectrométrique par le NADPH

Frucht- und Gemüsesäfte - Enzymatische Bestimmung der Gehalte an D-Glucose und D-Fructose - Spektralphotometrische Bestimmung von NADPH

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CEN

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

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Foreword

This European Standard has been prepared by the 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of an identical text or by endorsement, at the latest by April 1995, and conflicting national standards shall be withdrawn at the latest by April 1995.

Annexes designated "informative" are given only for information. In this standard annexes A and B are informative.

According to the CEN/CENELEC Internal Regulations, 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, United Kingdom.

1 Scope

This european standard specifies an enzymatic method for the determination of the D-glucose and D-fructose content 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.

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

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	β-Nicotinamide-adenine-dinucleotidephosphate ;
NADPH	β-Nicotinamide-adenine-dinucleotidephosphate (reduced form) ;
G-6-P	Glucose 6-phosphate ;
F-6-P	Fructose 6-phosphate ;
HK	Hexokinase (EC [1)] 2.7.1.1) ;
G6P-DH	Glucose-6-phosphate dehydrogenase (EC [1)] 1.1.1.49) ;
PGI	Phosphoglucose-isomerase (EC [1)] 5.3.1.9) ;
IU	One International Unit of enzyme activity, which catalyzes ; 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

D-glucose and D-fructose contained in a diluted test sample are phosphorylated in the C-6 position in an enzyme-catalysed reaction involving ATP and HK.

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

The D-fructose content may be determined by making use of a further reaction in which PGI catalyses the isomerization of F-6-P to G-6-P (4.3).

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

4.2 Reactions : D-glucose determination

$$\text{D-glucose} + \text{ATP} \xleftrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

4.3 Reactions : D-fructose determination

$$\text{D-fructose} + \text{ATP} \xleftrightarrow{\text{HK}} \text{F-6-P} + \text{ADP}$$

$$\text{F-6-P} \xrightarrow{\text{PGI}} \text{G-6-P}$$

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

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and only water in accordance with at least grade 3 of ISO 3696:1987.

5.2 Triethanolamine buffer, pH = 7,6

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

5.3 NADP solution

Dissolve 60 mg β-Nicotinamide-adeninedinucletoide phosphate-disodium salt (β-NADP-Na_2) in 6 ml water. The solution is stable for at least four weeks at 4 °C.

5.4 ATP solution

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

5.5 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 glucose-6-phosphate-dehydrogenase, ρ (G6P-DH) = 1 mg/ml, about 140 IU/ml with glucose 6-phosphate as substrate in ammonium sulfate solution,c ($(NH_4)_2SO_4$) = 3,2 mol/l. The suspension is stable for at least one year at 4 °C.

5.6 PGI enzyme suspension

Suspend phosphoglucose isomerase, ρ (PGI) = 2 mg/ml, about 700 IU/ml (with fructose 6-phosphate as substrate) in ammonium sulfate solution, c ($(NH_4)_2SO_4$) = 3,2 mol/l. The suspension is stable for at least one year at 4 °C.

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 glass or plastic, of 10 mm optical path length, and which do not have significant absorption at 334 nm, 340 nm and 365 nm.

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

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

7 Procedure

7.1 Preparation of the test sample

Normally products shall not be pretreated 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 high viscosity and/or very high content of cells (for example pulp), determination on the basis of a weighed test sample is the usual procedure.

Mix cloudy juices well. Dilute the test sample so that the D-glucose or the D-glucose + D-fructose concentration is between 0,1 g/l to 1,0 g/l. This solution is to be used for the determination even if it is coloured.

7.2 Test procedure

7.2.1 General

The determination shall normally be carried out at constant temperature between 20 °C and 25 °C. A constant temperature in the range 25 °C to 37 °C may also be used, providing equivalent results are obtained.

The absorption maximum of NADPH is at 340 nm. When using a variable wavelength spectrometer, measure at the absorption maximum only. When using a mercury vapour lamp, spectral-line photometer, measure at a wavelength of 334 nm or 365 nm.

Do not use single-mark transfer pipettes for pipetting the solutions. Solutions of enzyme, coenzyme and buffer may be added from suitable automatic pipettes. Enzyme test pipettes (6.1) or their equivalent (6.2) shall be used for pipetting the sample solution.

The determination may also be carried out using a commercially available test combination kit.

If the substance to be determined is available in a suitably pure form, it is recommended to include it as a standard solution.

7.2.2 Blank test solution

Pipette into cuvettes 1,00 ml buffer solution (5.2), 0,10 ml NADP solution (5.3), 0,10 ml ATP solution (5.4) and 2,00 ml water. Mix, and after about 3 min, read the absorbance (A_1) of the solution against air (no cuvette in lightpath).

7.2.3 Test sample solution

Pipette into cuvettes 1,00 ml buffer solution (5.2), 0,10 ml NADP solution (5.3), 0,10 ml ATP solution (5.4), 0,10 ml test sample and 1,90 ml water. Mix and after about 3 min, read the absorbance (A_1) of the solution against air (no cuvette in lightpath).

7.2.4 Enzyme reaction and quantification

The following procedure is carried out on each solution (7.2.2 and 7.2.3) individually :

Add 0,02 ml enzyme suspension (HK/G6P-DH ; 5.5). Mix, wait until the reaction has stopped (10 min to 15 min) and read the absorbances (A_2) of the solutions. Check for completion of reaction by reading absorbance A_2 at 2 min intervals. If the reaction is not complete after 15 min, extrapolate the absorbance back to the time of addition of enzyme suspension (HK/G6P-DH) (5.5).

Then add 0,02 ml enzyme suspension (PGI) (5.6). Mix, wait until the reaction has stopped (10 min to 15 min) and read the absorbances (A_3) of the solutions. Check the completion of reaction by reading absorbance A_3 at 2 min intervals. If the reaction is not complete after 15 min and the absorbance increase remains constant, extrapolate the absorbance back to the time of addition of enzyme suspension (PGI) (5.6).

8 Calculation

According to the reactions on which this determination is based, there is a linear proportionality between the amount of NADPH formed (and hence the absorbance difference ΔA) and the concentration of D-glucose and D-fructose.

$$\Delta A_{\text{D-glucose}} = (A_2 - A_1)_{\text{Sample}} - (A_2 - A_1)_{\text{Blank}}$$

$$\Delta A_{\text{D-fructose}} = (A_3 - A_2)_{\text{Sample}} - (A_3 - A_2)_{\text{Blank}}$$