SLOVENSKI STANDARD
SIST EN 12632:1999

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Sadni in zelenjavni sokovi – Encimska določitev vsebnosti ocetne kisline (acetata) – Spektrometrijska metoda z NAD

Fruit and vegetable juices - Enzymatic determination of acetic acid (acetate) content - NAD Spectrometric method

Frucht- und Gemüsesäfte - Enzymatische Bestimmung des Gehaltes an Essigsäure (Acetat) - Spektralphotometrische Bestimmung von NAD

Jus de fruits et de légumes - Dosage enzymatique de l'acide acétique (acétate) - Méthode spectrométrique par le NAD

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ICS:

67.160.20	Brezalkoholne pijače	Non-alcoholic beverages

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English version

Fruit and vegetable juices - Enzymatic determination of acetic acid (acetate) content - NAD Spectrometric method

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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 - 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of an identical text or by endorsement, at the latest by August 1999, and conflicting national standards shall be withdrawn at the latest by August 1999.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 an enzymatic method for the determination of the total content of acetic acid or acetate salts 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 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 accélaration due to gravity at the surface of the earth (9,81 m/s²).

3.2 Abbreviations

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

ACS Acetyl Coenzyme A synthetase ;

CoA Coenzyme A ;

ATP Adenosine-5'-Tri-phosphate ;

AMP Adenosine-Mono-phosphate ;

CS Citrate synthase ;

NAD β-Nicotinamide-adenine-dinucleotide ;

NADH β-Nicotinamide-adenine-dinucleotide, reduced form ;

MDH Malate-dehydrogenase ;

IU 1 International Unit (IU) of enzyme activity catalyses the conversion of 1 µmol of substance per minute at 25 °C under standard conditions.

4 Principle

Acetic acid (acetate) is converted in the presence of the enzyme acetyl-coenzyme A-synthetase (ACS) with adenosine-5'-triphosphate (ATP) and coenzyme A (CoA) to acetyl-CoA (reaction 1) :

$$\text{Acetate} + \text{ATP} + \text{CoA} \xrightarrow{\text{ACS}} \text{acetyl}-\text{CoA} + \text{AMP} + \text{pyrophosphate} \qquad (1)$$

Acetyl-CoA reacts with oxaloacetate to produce citrate in the presence of citrate synthase (CS) (reaction 2) :

$$\text{Acetyl-CoA} + \text{oxaloacetate} + H_2O \xrightarrow{\text{CS}} \text{citrate} + \text{CoA} \qquad (2)$$

The oxaloacetate required for reaction (2) is formed from malate and nicotinamide-adenine dinucleotide (NAD) in the presence of malate dehydrogenase (MDH) (reaction 3). In this reaction NAD is reduced to NADH.

$$\text{malate} + NAD^+ \xrightarrow{\text{MDH}} \text{oxaloacetate} + \text{NADH} + H^+ \qquad (3)$$

The determination is based on the formation of NADH measured by the increase in absorbance at 340 nm, 334 nm or 365 nm. Since a preceding indicator reaction is used, the amount of NADH formed is related to the acetic acid concentration but not linearly proportional.

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.

NOTE : The determination can also be carried out using a commercially available test kit.

5.2 Triethanolamine hydrochloride

5.3 L-malic acid.

5.4 Magnesium chloride, $MgCl_2.6H_2O$.

5.5 Potassium hydroxide solution, c(KOH) = 2 mol/l.

5.6 Nicotinamide-adenine dinucleotide (*ca.* 98 %).

5.7 Coenzyme A

5.8 Adenosine-5'-triphosphate, disodium salt ATP-Na_2H_2.

5.9 Sodium hydrogen carbonate, $NaHCO_3$.

5.10 Malate dehydrogenase, suspension in ammonium sulfate, c(MDH) = 3,2 mol/l, specific activity approximately 1 200 IU/mg.

5.11 Citrate synthase, suspension in ammonium sulfate, c(CS) = 3,2 mol/l, specific activity approximately 110 IU/mg.

5.12 Acetyl-CoA synthetase, lyophilized.

5.13 Ammonium sulfate, $(NH_4)_2SO_4$.

5.14 Sodium acetate, $CH_3COONa.3H_2O$.

5.15 Buffer solution, pH = 8,4.

Dissolve 7,59 g of triethanolamine hydrochloride (5.2), 420 mg of malic acid (5.3) and 210 mg of magnesium chloride (5.4) in approximately 70 ml of water. Adjust the solution pH to 8,4 with approximately 21 ml of the potassium hydroxide solution (5.5) and make up to 100 ml with water. The buffer is stable for 4 weeks at + 4 °C.

5.16 Nicotanimide-adenine dinucleotide/Coenzyme A - solution

Dissolve 144 mg of NAD (5.6) and 30 mg of CoA (5.7) in 6 ml of water. The solution is stable for 1 week at + 4 °C.

5.17 Adenosine-5'-triphosphate solution

Dissolve 300 mg of ATP-Na_2H_2 (5.8) and 300 mg of sodium hydrogen carbonate (5.9) in 6 ml of water. The solution is stable for 4 weeks at + 4 °C.

5.18 Malate dehydrogenase/Citrate synthase - suspension

Mix 0,6 ml of the MDH suspension (5.10) and 0,6 ml of the CS suspension (5.11). The suspension is stable for 1 year at + 4 °C.

5.19 Acetyl-CoA synthetase suspension

Dissolve 20 mg of the ACS lyophilizate (5.12) in 0,5 ml of the ammonium sulfate solution (5.20). The suspension is stable for 2 weeks at + 4 °C.

5.20 Ammonium sulfate solution, $c((NH_4)_2SO_4) = 1$ mol/l.

Dissolve 13,2 g of ammonium sulfate (5.13) in approximately 80 ml of water, adjust to pH 7,3 with approximately 0,2 ml of potassium hydroxide solution (5.5) and make up to 100 ml with water. The solution is stable for 1 year at 20 °C to 25 °C.

5.21 Acetate standard solution, $c(CH_3COO^-) = 5$ mmol/l.

Dissolve 68 mg of sodium acetate (5.14) in 100 ml of water. Prepare fresh solution before use.

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 an accuracy equivalent to 6.1 (alternative to 6.1) for example positive displacement capillary pipettes.

6.3 Cuvettes, made of quartz, glass or plastic, of 1 cm optical path length, which do not have a 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 Spectrophotometer, (variable wavelength) for measuring at 340 nm (alternative to 6.4).

6.6 Centrifuge, capable of producing a centrifugal acceleration of 3 000 *g* at the base of the centrifuge tube (6.8).

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

$$a = 11{,}18 \times r \times (n/1000)^2 \quad (4)$$