SLOVENSKI STANDARD
SIST EN 12631:1999

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Sadni in zelenjavni sokovi – Encimska določitev vsebnosti D in L mlečne kisline (laktata) – Spektrometrijska metoda z NAD

Fruit and vegetable juices - Enzymatic determination of D- and L-lactic acid (lactate) content - NAD spectrometric method

Frucht- und Gemüsesäfte - Enzymatische Bestimmung des Gehaltes an D- und L-Milchsäure (Lactat) - Spektralphotometrische Bestimmung von NAD

Jus de fruits et de légumes - Dosage enzymatique des acides D- et L-lactiques (lactate) - 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 D- and L-lactic acid (lactate) 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of an identical text or by endorsement, at the latest by August 1999, and conflicting national standards shall be withdrawn at the latest by August 1999.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 D- and L-lactic acid and lactate 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 ;
ϕ	volume fraction ;
g	acceleration 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 :

GPT	Glutamate-pyruvate-transaminase ;
L-LDH	L-lactate dehydrogenase ;
D-LDH	D-lactate dehydrogenase ;
NAD	β-Nicotinamide-adenine-dinucleotide ;
NADH	β-Nicotinamide-adenine-dinucleotide, reduced form ;
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

L-lactic acid (lactate) is oxidized by nicotinamide-adenine-dinucleotide (NAD) in the presence of L-lactate dehydrogenase (L-LDH) to produce pyruvate. D-lactic acid (lactate) is oxidized in the same way in the presence of D-lactate dehydrogenase (D-LDH).

$$\text{L-lactate} + NAD^+ \overset{\text{L-LDH}}{\Leftrightarrow} \text{pyruvate} + NADH + H^+ \qquad (1)$$

$$\text{D-lactate} + NAD^+ \overset{\text{D-LDH}}{\Leftrightarrow} \text{pyruvate} + NADH + H^+ \qquad (2)$$

The equilibrium of the reactions (1) and (2) lies almost completely on the side of lactate. However, by trapping the pyruvate in a subsequent reaction catalysed by the enzyme glutamate-pyruvate transaminase (GPT) in the presence of L-glutamate (equation 3), the equilibrium can be displaced in favour of pyruvate and NADH.

$$\text{Pyruvate} + \text{L-glutamate} \overset{\text{GPT}}{\Leftrightarrow} \text{L-alanine} + \alpha\text{-ketoglutarate} \qquad (3)$$

The amount of NADH formed (as measured by the increase in absorbance at 334 nm, 340 nm or 365 nm) is equivalent to the amounts of D- and L-lactic acid present in the sample.

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 combination kit.

5.2 Glycylglycine

5.3 L-glutamic acid

5.4 Sodium hydroxide solutions

5.4.1 Sodium hydroxide solution, $c(NaOH) = 10$ mol/l.

5.4.2 Sodium hydroxide solution, $c(NaOH) = 10$ mmol/l.

5.5 Nicotinamide-adenine dinucleotide

5.6 Glutamate-pyruvate transaminase, suspension, ρ(GPT) = 10 mg/l, specific activity of approximately 80 IU/mg.

5.7 L-Lactate dehydrogenase, solution in glycerol ϕ(L-LDH) = 50 %, specific activity of approximately 550 IU/mg.

5.8 D-Lactate dehydrogenase, suspension in ammonium sulfate, c(D-LDH) = 3,2 mol/l, specific activity of approximately 300 IU/mg.

5.9 L-Lactic acid standard solution, c(L-lactic acid) = 1,0 mol/l.

5.10 D-Lactate, Lithium salt

5.11 Buffer solution, pH = 10,0

Dissolve 4,75 g of glycylglycine (5.2) and 0,88 g of L-glutamic acid (5.3) in approximately 50 ml of water. Adjust to pH 10,0, with approximately 4,6 ml of sodium hydroxide (5.4.1) and make up to 60 ml with water. The solution is stable for at least 3 months at + 4 °C.

5.12 Nicotinamide-adenine dinucleotide solution

Dissolve 420 mg of NAD (5.5) in 12 ml of water. The solution is stable for at least 4 weeks at + 4 °C.

5.13 Glutamate-pyruvate transaminase suspension

Centrifuge 2 ml of the GPT-suspension (5.6) for 10 min at approximately 4 000 rpm. Aspirate and discard 1,0 ml of the clear supernatant, shake the suspension well prior to use. The suspension is stable for at least one year at + 4 °C.

5.14 L-Lactate dehydrogenase solution

Use the L-LDH solution (5.7) undiluted. The solution is stable for at least one year at + 4 °C.

5.15 D-Lactate dehydrogenase suspension

Use the D-LDH suspension (5.8) undiluted. The suspension is stable for at least one year at + 4 °C.

5.16 L-Lactic acid standard solution, c($CH_3CHOHCOOH$) = 0,5 mmol/l.

Dilute L-lactic acid standard solution (5.9) 1 to 2 000 with sodium hydroxide (5.4.2). Prepare fresh solution prior to use.

5.17 D-Lactic acid standard solution, c($CH_3CHOHCOOLi$) = 0,5 mmol/l.

Dissolve 48 mg of D-lactate, Li-salt (5.10) in 100 ml water. Dilute this solution 1 volume part to 9 volume parts with water. Prepare fresh solution prior to 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 :

$$a = 11{,}18 \times r \times (n/1\,000)^2 \qquad (4)$$

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 rotational frequency per minute.