

Fruit and vegetable juices — Determination of free amino acids content — Liquid chromatographic method

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British Standard

ICS 67.160.20

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Summary of pages

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English version

Fruit and vegetable juices — Determination of the free amino acids content — Liquid chromatographic method

Jus de fruits et de légumes — Dosage des teneurs en acides aminés libres — Méthode par chromatographie en phase liquide

Frucht- und Gemüsesäfte — Bestimmung der Gehalte an freien Aminosäuren — Flüssigchromatographisches Verfahren

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of an identical text or by endorsement, at the latest by August 1999, and conflicting national standards shall be withdrawn at the latest by August 1999.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

Contents

	Page
Foreword	2
1 Scope	3
2 Normative references	3
3 Symbols	3
4 Principle	3
5 Reagents	3
6 Apparatus	3
7 Procedure	4
8 Calculation	4
9 Precision	5
10 Test report	5
Annex A (informative) Bibliography	6
Annex B (informative) Statistical results of the inter-laboratory test	6
Annex C (informative) Details of typical conditions and reagents used in the analysis of free amino acids in fruit and vegetable juices	9

1 Scope

This European Standard specifies a chromatographic procedure for the determination of the free amino acid content 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

For the purpose of this European Standard, the following symbols apply:

- c substance concentration;
- ρ mass concentration;
- g acceleration due to gravity at the surface of the earth (9,81 m/s²).

4 Principle

Amino acids are separated on a cation exchange chromatography column by stepwise elution with a range of lithium citrate buffers of different molarities and pH values. After a colour reaction, with ninhydrin, the amino acids are quantified photometrically at a wavelength of 570 nm (or 440 nm for proline).

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and only water in accordance with at least grade 1 of EN ISO 3696:1995.

5.2 Buffer solutions for amino acid elution

Prepare the buffer solutions in accordance with the manufacturer's instructions. Ready-prepared lithium citrate buffer of different pH values and lithium ion molarities can also be used.

The molarities and pH values of buffer solutions can differ in accordance with the equipment manufacturer's instructions (e.g. the length and diameter of the analytical column and type of resin). Therefore it is impossible to give exact details for the preparation of the various buffer solutions. The description of the buffer solutions detailed in annex C are therefore only for use as a guide for the user of this standard.

5.3 Lithium hydroxide solution $c(\text{LiOH}) = 0,2 \text{ mol/l}$ to $0,4 \text{ mol/l}$

An example for the preparation of this solution for column regeneration is given in annex C.

5.4 Colour reagent

Ninhydrin in a solution of 2-methoxyethanol and sodium acetate buffer. The stability of this mixture is very variable and ranges from a few days to a few weeks.

An example for the preparation of the solution used in the colour development stage of this process is given in annex C.

5.5 Lithium citrate buffer (pH = 2,00 to 2,20) for sample dilution

An example for the preparation of this dilution buffer is given in annex C.

5.6 Standard amino acids

Weigh out individual portions of amino acids so that, after appropriate dilution, the absolute levels in the analyser fall within the linear range for each amino acid. It is useful to use a standard solution of similar concentration to that expected in the sample. The use of certified reference materials¹⁾ is recommended for this procedure. An example for the preparation of the amino acid test solution is given in annex C.

6 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.1 Amino acid analyser, with one of the following column dimensions:

9 mm × 500 mm, 6 mm × 200 mm, 4,6 mm × 200 mm
or 270 mm, 4 mm × 200 mm, 4 mm × 100 mm
or 3,2 mm × 140 mm

The columns can be made of glass or steel. The various columns, described above, give significant differences in peak resolution and analysis times.

¹⁾ These materials can be supplied by the Institute for reference materials and measurement, C.C.R Retieseweg, 2400 Geel, Belgium. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.