SLOVENSKI STANDARD
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Sadni in zelenjavni sokovi - Določevanje prostih amino kislin – Metoda s tekočinsko kromatografijo

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

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

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

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English version

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

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

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Contents

Page

Foreword 3
1 Scope 4
2 Normative references 4
3 Symbols 4
4 Principle 4
5 Reagents 4
6 Apparatus 5
7 Procedure 7
8 Calculation 8
9 Precision 8
10 Test report 9
Annex A (informative) Bibliography 10
Annex B (informative) Statistical results of the inter-laboratory test 11
Annex C (informative) Details of typical conditions and reagents used in the analysis of free amino acids in fruit and vegetable juices 13

1 Scope

This draft 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 draft 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 draft 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.

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.

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(LiOH) = 0,2 mol/l to 0,4 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 pH = 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 materials [1)] 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 :

[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.

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 x 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.

6.2 Chromatographic resins (cation exchanger)

The resins are only available under trade names and are specifically adapted for the various commercial analysers (e.g. Durrum [2] DC-6a, Biotronik [2] BTC 3118, Biotronik [2] BTC 2710, LKB Ultropac [2] II, LKB Ultropac [2] 8, 6300/7300 Beckman [2]). Pre-columns are also available for a range of these resins to remove traces of ammonium salts from the buffer solutions and to help prolong the effective life of the analytical columns (Examples are : Durrum [2] C-3, Biotronik [2] BTC-F, LKB Ultropac [2] I).

6.3 Centrifuge, capable of producing a centrifugal force of 4 000 *g* at the base of the centrifuge tube (6.4).

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

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

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.

6.4 Centrifuge tubes

6.5 Membrane filter, non sterile hydrophilic syringe filter(s) with a pore size of 0,45 μm.

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[2] Durrum, Biotronik, Beckman & Ultropac are trade names. This information is given for the convenience of users of this standard and does not constitute an endorsemnt by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

7 Procedure

7.1 Preparation of the test sample

Normally products shall not be pre-treated and their analysis by this method shall be on a volumetric basis, results being expressed per litre of sample. The analysis of concentrated samples 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 cells (for example pulp), determination on the basis of a weighed test sample is the usual procedure.

Mix cloudy samples well before dilution.

Mix the juice sample with the dilution buffer (5.5) and the internal standard solution (c(nor-leucine or nor-valine) = 1 mmol/l, as detailed in annex C). Centrifuge this solution at 4 000 g for 15 min. Finally clarify the sample by passing the solution through the syringe filter (6.5) and the sample is ready for analysis.

NOTE : The sample dilution required depends on the expected composition of the juice and loading mixture. However, this normally lies between 1 : 1 and 1 : 10. For orange juice a dilution of 1 : 10 is typically used. In special cases, e.g. cherry juice, it is recommended that two separate dilutions be used (for example 1 : 5 and 1 : 20). This allows for the wide variation in the concentrations of the various amino acids (for example in the case of cherry the very high level of asparagine).

The sample loading varies from one system to another and also depends on the sample dilution. However, this is generally between 20 μl to 300 μl.

7.2 Test procedure

Carry out the amino acid separation according to the particular instrumental conditions. Elution with 4 to 6 different buffers (5.2) of different pH values and ionic strengths can be used. For example a normal initial buffer would have a lithium ion concentration of c(LiOH) = 0,10 mol/l and a pH of 2,94 (buffer A in annex C). This buffer strength would typically increase to c(LiOH) = 1,40 mol/l and this buffer would have a pH of 3,65 (buffer E in annex C). Maintain the column at a constant temperature between approximatively 30 °C and 80 °C dependent on the elution step carried out and on the actual system employed. Choose the pH, lithium molarity, the running time of the different buffer and the column temperature to give the optimum separation conditions, which may vary from one laboratory to another.

Regenerate the column with a lithium hydroxide solution (c(LiOH) = 0,2 mol/l to 0,4 mol/l) between samples and prior to re-equilibration of the column in the initial buffer run.

The temperature at which the colour reaction, with ninhydrin, is carried out is usually between 100 °C and 130 °C. The reaction products are measured photometrically at a wavelength of 570 nm for all the amino acids except proline which is detected at 440 nm.