SLOVENSKI STANDARD
SIST ENV 12140:1998

01-junij-1998

Sadni in zelenjavni sokovi - Določevanje razmerja stabilnih ogljikovih izotopov (13C/ 12C) v sladkorjih iz sadnih sokov - Metoda masne spektrometrije razmerja izotopov

Fruit and vegetable juices - Determination of the stable carbon isotope ratio (13C/12C) of sugars from fruits juices - Method using isotope ratio mass spectrometry

Frucht- und Gemüsesäfte - Bestimmung des Verhältnisses der stabilen Kohlenstoff-Isotope (13C/12C) im Zuckeranteil von Fruchtsäften - Verfahren unter Verwendung der Isotopenverhältnis-Massenspektrometrie

Jus de fruits et de légumes - Détermination du rapport des isotopes stables du carbone (13C/12C) des sucres contenus dans les jus de fruits - Méthode utilisant la spectrométrie de masse des rapports isotopiques

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67.160.20	Brezalkoholne pijače	Non-alcoholic beverages

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English version

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CEN

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

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Contents

Foreword

This European Prestandard has been prepared by the Technical Committee CEN/TC 174 "Fruit and vegetable juices - Methods of analysis" of which the secretariat is held by AFNOR.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are required to announce the existence of this European Prestandard : Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Prestandard specifies a method for the determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruit juices by isotope ratio mass spectrometry (IRMS).

2 Normative references

This European Prestandard incorporates by dated or undated reference, provisions from other publications. These normatives 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 Prestandard 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 5725:1986	Precision of the test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests

3 Symbols

For the purposes of this standard the following symbols apply :

$(^{13}C/^{12}C)$	Isotope ratio of carbon 13 to carbon 12 for a considered sample ;
$\delta^{13}C$	Carbon 13 (^{13}C) content expressed in parts per thousand (‰);
g	Acceleration due to gravity at the surface of the earth (9,81 m/s^2) ;

4 Principle

$^{13}C/^{12}C$ isotope ratio in the carbon dioxide obtained from total and careful combustion of the sugars is determined by an isotope ratio mass spectrometer.

5 Reagents

Use only reagents of recognized analytical grade and only water in accordance with at least grade 3 of EN ISO 3696:1995.

5.1 Calcium hydroxide

5.2 Sulfuric acid, 95 % to 97 % (*m/m*)

6 Apparatus

Usual laboratory apparatus and, in particular, the following :

6.1 Isotope ratio mass spectrometer, with the ability to determine the ^{13}C content of CO_2 gas at natural abundance with an internal precision of 0,05 ‰ or better (expressed in relative δ value (see 8)). The internal precision is here defined as the difference between two measurements of the same CO_2 sample.

The mass spectrometer will generally be fitted with a triple collector to simultaneously register at mass numbers 44, 45 and 46. The mass spectrometer should either be fitted with a dual inlet system, for alternatively measuring the unknown sample and a standard, or use an on-line system which combusts the sample in an elemental analyser (6.2) followed by GC separation of the combustion products prior to isotopic mass spectrometric determination. The former method offers the highest accuracy for the determination of variations in the isotope contents in the range of the natural abundance. However, correct results can also be obtained using the on-line method provided a secondary standard is used.

6.2 Combustion apparatus (elemental analyser), which can quantitatively convert all carbon of the sample into carbon dioxide (CO_2), and which is able to remove all other combustion products mainly water from the CO_2.

6.3 Centrifuge, capable of producing a centrifugal acceleration of 1400 *g* at the base of the centrifuge tube (6.4).

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

$$a = 11{,}18 \times r \times (n / 1000)^2 \qquad (1)$$

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, of 50 ml capacity

7 Procedure

7.1 Preparation of the test sample

Remove the solid constituent of a sample of approximately 50 ml of natural or reconstituted fruit juice by centrifugation (6.3), at 1400 *g* for 10 min.

7.2 Purification and separation of sugars

Purify the soluble substances remaining in the supernatant liquid after centrifugation by the addition of 2 g of powdered calcium hydroxide (5.1) to the solution whilst stirring it well (using, for example a magnetic stirrer) and heating in a water bath at 90 °C for 3 min.

During this stage of the procedure, organic acids, amino acids and other compounds are precipitated. Separate the precipitate by centrifugation (6.3) of the hot solution (for 3 min at 1 400 *g*). Decant the clear supernatant liquid and acidify it with 0,1 mol/l sulfuric acid (5.2) in order to obtain a pH of approximately 5 when the colour of the solution changes. This solution contains mainly sugars, calcium sulfate and some colorants as minor ingredients. Partially remove residual calcium sulfate by storing the solution in a refrigerator at approximately 4 °C overnight (approximately 15 h) followed by decantation. Freeze-dry the supernatant liquid and homogenize the lyophilisate to a fine powder before storing it in a glass vial with an air-tight plastics cap.

7.3 Combustion of sugars

Combust the sample obtained using the procedure given in 7.2 in a circulating oxygen gas stream or in an elemental analyser (6.2). It is essential to effect complete conversion of organic carbon into carbon dioxide by a method that avoids any isotopic fractionation and allows the collection of the gas as a whole. A liquid nitrogen trap is usually employed to collect the carbon dioxide before analysis by isotope ratio mass spectrometry.

NOTE : Suitable microcombustion systems are commercially available.

7.4 Determination

The $^{13}C/^{12}C$ isotope ratio in the carbon dioxide obtained from combustion of the sugars, as given in 7.3, is determined with the aid of an isotope ratio mass spectrometer (6.1). Determine the ratio for the isotopic species $^{13}CO_2/^{12}CO_2$ from the corresponding intensities.

8 Calculation

In addition to the commonly used mass isotopic abundance (in ‰ of atoms), the so-called delta value (δ) is also used as an alternative system of units for indicating isotope content. Delta values are used exclusively for indicating variations (of the third decimal place) in the natural isotopic abundance.

Express the $\delta^{13}C$ values as the relative difference per thousand between the ^{13}C and ^{12}C ratios of a sample in relation to a standard, Pee Dee Belemnite from South Carolina in USA (the PDB standard). This is a fossil calcium carbonate with an isotope ratio $(^{13}C/^{12}C)$ PDB = 0,011 237 2 for the emitted CO_2. This value is the reference point of the common international PDB scale for $\delta\ ^{13}C$ values expressed in parts per thousand (‰) which are calculated using the following equation :

$$\delta^{13}C_{PDB} = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}}{(^{13}C/^{12}C)_{PDB}} \times 1\,000 \qquad (2)$$

A suitable secondary standard for routine use in this method is NBS 22 (obtained from International Atomic Energy Agency (IAEA)[1)]), which has a value of - 29,80 ‰ relative to PDB.

9 Precision

Details of the interlaboratory test on precision of the method are summarized in annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in annex B.

9.1 Repeatability

The absolute difference between two single results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

The values are :

Orange juice	r = 0,26 ‰;
Pineapple juice	r = 0,42 ‰;
Beet sugar	r = 0,17 ‰;
Cane sugar	r = 0,29 ‰.

9.2 Reproducibility

The absolute differences between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values are :

Orange juice	R = 0,66 ‰;
Pineapple juice	R = 0,72 ‰;

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