SLOVENSKI STANDARD
SIST ENV 13070:1999

01-maj-1999

Sadni in zelenjavni sokovi - Določevanje razmerja stabilnih izotopov ogljika (13C/12C) v pulpi sadnih sokov - Metoda z uporabo izotopskega razmerja - masna spektrometrija

Fruit and vegetable juices - Determination of the stable carbon isotope ratio (13C/12C) in the pulp of fruit juices - Method using isotope ratio mass spectrometry

Frucht- und Gemüsesäfte - Bestimmung des Verhältnisses der stabilen Kohlenstoff-Isotope (13C/12C) in der Pulpe von Fruchtsäften - Verfahren unter Anwendung der Isotopenverhältnis-Massenspektroskopie

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

Ta slovenski standard je istoveten z: **ENV 13070:1998**

ICS:

67.160.20	Brezalkoholne pijače	Non-alcoholic beverages

SIST ENV 13070:1999 **en**

EUROPEAN PRESTANDARD
PRÉNORME EUROPÉENNE
EUROPÄISCHE VORNORM

ENV 13070

May 1998

ICS 67.160.20

Descriptors: fruit and vegetable juices, pulps, chemical analysis, determination, ratios, isotopes, carbon, mass spectrometry

English version

Fruit and vegetable juices - Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) in the pulp of fruit juices - Method using isotope ratio mass spectrometry

Jus de fruits et de légumes - Détermination du rapport des isotopes stables du carbone ($^{13}C/^{12}C$) dans la pulpe des jus de fruits - Méthode utilisant la spectrométrie de masse des rapports isotopiques

Frucht- und Gemüsesäfte - Bestimmung des Verhältnisses der stabilen Kohlenstoff-Isotope ($^{13}C/^{12}C$) in der Pulpe von Fruchtsäften - Verfahren unter Anwendung der Isotopenverhältnis-Massenspektroskopie

This European Prestandard (ENV) was approved by CEN on 9 April 1998 as a prospective standard for provisional application.

The period of validity of this ENV is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the ENV can be converted into a European Standard.

CEN members are required to announce the existence of this ENV in the same way as for an EN and to make the ENV available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the ENV) until the final decision about the possible conversion of the ENV into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Contents

Foreword 3
1 Scope 4
2 Normative references 4
3 Symbols 4
4 Principle 4
5 Reagents 4
6 Apparatus 5
7 Procedure 6
8 Calculation 7
9 Precision 7
10 Test report 8
Annex A (informative) Bibliography 9
Annex B (informative) Statistical results of the interlaboratory test 10

Foreword

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this European Prestandard: 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 Prestandard specifies a method for the determination of the stable carbon isotope ratio of the pulp in fruit juices. The determination of this parameter is useful as an internal standard for comparison with the Carbon 13 content value ($\delta^{13}C$) obtained for the sugars.

2 Normative references

This European Prestandard 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 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 test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests
ENV 12140:1996	Fruit and vegetable juices - Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruit juices - Method using isotope ratio mass spectrometry

3 Symbols

For the purpose of this standard the following symbols apply :

$(^{13}C/^{12}C)$	Isotope ratio of carbon 13 to 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

The carbon contained in the pulp is quantitatively combusted into carbon dioxide. The ratio of the two stable carbon isotopes (^{13}C and ^{12}C) is then measured using a isotope ratio mass spectrometer. This value can then be compared with the value obtained for the sugars (as determined using ENV 12140). If there is too large a difference between the value in the pulp and the sugars this can indicate the addition of sugars derived from a C_4 plant such as cane or corn.

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)

5.3 Acetone

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 a precision of 0,05 % or better expressed as relative δ value (see 8) (one measuring cycle with ten integration periods).

The mass-spectrometer will generally be fitted with a triple collector to simultaneous register at mass number 44, 45 and 46. The mass spectrometer should be fitted either with a dual inlet system, for alternately 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 gas chromatographic separation of the combustion products prior to isotopic mass spectrometric determination. The former method offers the highest accuracy for the determination of variations of the isotope content 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 converts all the carbon of the sample into carbon dioxide (CO_2), and which is able to convert all nitrous oxides formed, during the oxidation process, into nitrogen gas and to remove all other combustion products, including water, from the CO_2.

6.3 Centrifuge, capable of producing a centrifugal acceleration of 1 400 *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 :

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

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, of 50 ml capacity.

7 Procedure

7.1 Preparation of the test sample

50 ml of fruit juice (natural or reconstituted) is centrifuged at 1 400 *g* for 10 min to separate the uspended solids (pulp). To remove any adhering sugars the centrifugate is washed with water. To achieve this the pellet is resuspended in 40 ml of water and stirred for 5 min. This suspension is then recentrifuged and the water discarded. A fresh aliquot of water is added and the process is repeated. A further aliquot of water is used in the third and final wash with water.

After this third wash, with water, the pulp is resuspended in 40 ml of acetone to extract any lipid material. After stirring in acetone the pulp is centrifuged as given above and the pulp separated from the solvent. A fresh aliquot of acetone is added and the process repeated twice more.

To obtain meaningful results it is essential that both the water and acetone washes are carried thoroughly. This ensures that all the occluded sugars and lipid materials are removed. If the pulp is not properly washed the lipid material will cause a significant negative shift in the $\delta^{13}C$ content of the pulp.

The pulp after washing, as described above, is finally dried under vacuum which gives a white/gray solid which can be easily homogenised.

7.2 Combustion of pulp

Combust the samples obtained using the procedures given in 7.1 using the procedure for sugars described in ENV 12140:1996.

Care should be taken to ensure that there is a quantitative reduction of all the nitrous oxides formed, from the nitrogen containing compounds in the pulp, during the combustion stage. If this is not achieved nitrous oxide will be present in the carbon dioxide gas and this will contribute to the intensity of the mass 46 ion. This will lead to an incorrect correction for oxygen 17 and so give a too low carbon 13 content of the pulp.

NOTE : Suitable microcombustion systems are commercially available.

7.3 Determination

The $^{13}C/^{12}C$ isotope ratio in the carbon dioxide obtained from the combustion of the pulp, as described in 7.2, 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 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 (on 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, the Pee Dee Belemnite from South Carolina-USA (the PDB standard). This is a fossil calcium carbonate with an isotope ratio $(^{13}C/^{12}C)_{PDB} = 0{,}0112372$ 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)], Vienna), which has a $\delta^{13}C$ value of - 29,80 ‰ relative to PDB.

9 Precision

Details of the interlaboratory test on the precision of the method are summarized in annex B. The values derived from interlaboratory test may not be applicable to analysis of concentration ranges and matrices other than those 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 $r = 0{,}32$ ‰ ;
- orange juice with added sugar $r = 0{,}31$ ‰ ;
- grapefruit juice $r = 0{,}26$ ‰ ;
- grapefruit juice with added sugar $r = 0{,}45$ ‰ ;
- pineapple juice $r = 0{,}42$ ‰ ;
- pineapple juice with added sugar $r = 0{,}46$ ‰.

1) International Atomic Energy Agency P.O. Box 100 A-1400 Wien - Austria.