DRAFT FOR DEVELOPMENT

DD ENV
12140:1997

Method for

Determination of stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruit juices, using isotope ratio mass spectrometry

ICS 67.160.20

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Committees responsible for this Draft for Development

The preparation of this Draft for Development was entrusted to Technical Committee AW/21, Fruit and vegetable juices, upon which the following bodies were represented:

British Fruit Juice Importers' Association
British Retail Consortium
British Soft Drinks Association Limited
Campden and Chorleywood Food Research Association
Department of Trade and Industry (Laboratory of the Government Chemist)
Leatherhead Food Research Association
Ministry of Agriculture, Fisheries and Food
Royal Society of Chemistry

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National foreword

This Draft for Development has been prepared by Technical Committee AW/21, and is the English language version of 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,* published by the European Committee for Standardization (CEN).

ENV 12140:1996 was produced as a result of international discussions in which the United Kingdom took an active part.

This publication is not to be regarded as a British Standard

It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion to a European Standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its 3-year life. The commencement of the review period will be notified by an announcement in *BSI Update.*

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the prestandard or to withdraw it. Comments should be sent in writing to the Secretary of BSI Technical Committee AW/21 at BSI, 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

Cross-references

Publication referred to	Corresponding British Standard
ISO 3696:1987	BS EN ISO 3696:1995 *Specification for water for laboratory use*

ISO 5725:1986, to which informative reference is made in the text, has been superseded by ISO 5725-1:1994, ISO 5725-2:1994, ISO 5725-3:1994, ISO 5725-4:1994 and ISO 5725-6:1994, which are identical with the following Parts of BS ISO 5725 *Accuracy (trueness and precision) of measurement methods and results:*

BS ISO 5725-1:1994, *General principles and definitions.*

BS ISO 5725-2:1994, *Basic method for the determination of repeatability and reproducibility of a standard measurement method.*

BS ISO 5725-3:1994, *Intermediate measures of the precision of a standard measurement method.*

BS ISO 5725-4:1994, *Basic method for the determination of the trueness of a standard measurement method.*

BS ISO 5725-6:1994, *Use in practice of accuracy values.*

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the ENV title page, pages 2 to 8, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

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EUROPEAN PRESTANDARD
PRÉNORME EUROPÉENNE
EUROPÄISCHE VORNORM

ENV 12140

October 1996

ICS 67.160.20

Descriptors: Food products, beverages, fruit and vegetable juices, chemical analysis, determination, ratios, isotopes, carbon mass spectrometry

English version

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

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

Frucht- und Gemüesäfte — Bestimmung des Verhältnisses der stabilen Kohlenstoff-Isotope ($^{13}C/^{12}C$) im Zuckeranteil von Fruchtsäften — Verfahren unter Verwendung der Isotopenverhältnis-Massenspektrometrie

This European Prestandard (ENV) was approved by CEN on 1996-02-29 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 an European Standard (EN).

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

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Ref. No. ENV 12140:1996 E

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.

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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.

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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:

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

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 1 400 *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.

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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 ‰;
Beet sugar	R = 0,87 ‰;
Cane sugar	R = 0,60 ‰.

10 Test report

The test report shall contain the following data:

— all information necessary for the identification of the sample (kind of sample, origin of sample, designation);

— a reference to this European Standard;

— the date and type of sampling procedure (if known);

— the date of receipt;

[1)] International Atomic Energy Agency
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— the date of test;

— the test results and units in which they have been expressed;

— whether the repeatability of the method has been verified;

— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional, which might have affected the results.

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Annex A (informative)
Bibliography

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Documents CEN/TC 174/WG1 N 4 and N 5, Association Française de Normalisation, Paris-la Défense, 1989.

Annex B (informative)
Statistical results of the interlaboratory test

In accordance with ISO 5725:1986, the following parameters have been defined in an interlaboratory test. (For literature pertaining to the method see annex A). The test was conducted by the Working Group 1 "Isotope" of the CEN/TC 174.

Year of the interlaboratory test	1992
Number of laboratories	15
Number of samples	4

Table B.1

Sample	A	B	C	D
Number of laboratories retained after eliminating outliers	15	15	14	15
Number of outliers (laboratories)	0	0	1	0
Number of accepted results	108	103	54	56
Mean value ($\bar{x}$) (‰)	– 24,60	– 12,10	– 25,60	– 11,20
Repeatability standard deviation (S_r) (‰)	0,09	0,15	0,06	0,10
Repeatability relative standard deviation (RSD_r) (%)	0,37	1,24	0,23	0,89
Repeatability limit (r) (‰)	0,26	0,42	0,17	0,29
Reproducibility standard deviation (s_R) (‰)	0,21	0,21	0,30	0,18
Reproducibility relative standard deviation (RSD_R) (%)	0,85	1,74	1,17	1,61
Reproducibility limit (R) (‰)	0,66	0,72	0,87	0,60
NOTE There was no dependency detected between r, R and $\bar{x}$.				

Sample types:

A orange juice;

B pineapple juice;

C beet sugar;

D cane sugar.

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List of references

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