SLOVENSKI STANDARD
kSIST-TP FprCEN/TR 17674:2021

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Bioizdelki - Uporaba stabilnih razmerij izotopov ogljika, vodika, kisika in dušika kot orodij za preverjanje izvora biosurovin in karakteristik proizvodnih procesov - Pregled ustrezne obstoječe uporabe

Bio-based products- Use of stable isotope ratios of Carbon, Hydrogen, Oxygen and Nitrogen as tools for verification of the origin of bio-based feedstock and characteristics of production processes - overview of relevant existing applications

Biobasierte Produkte - Verwendung der Verhältnisse stabiler Isotope von Kohlenstoff, Wasserstoff, Sauerstoff und Stickstoff als Werkzeuge zur Überprüfung der Herkunft von biobasierten Rohstoffen und der Eigenschaften von Produktionsprozessen - Übersicht über relevante bestehende Anwendungen

Produits biosourcés - Utilisation des rapports isotopiques stables du carbone, de l'hydrogène, de l'oxygène et de l'azote comme outils de vérification de l'origine des matières premières biosourcées et des caractéristiques des procédés de production - Vue d'ensemble des applications existantes pertinentes

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This draft Technical Report is submitted to CEN members for Vote. It has been drawn up by the Technical Committee CEN/TC 411.

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

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European foreword

This document (FprCEN/TR 17674:2021) has been prepared by Technical Committee CEN/TC 411 “Bio-based products”, the secretariat of which is held by NEN.

This document is currently submitted to the Vote on TR.

Introduction

Part of **OPEN BIO Deliverable N°3.8** is used as starting point for the description given in this document. Bio-based products from forestry and agriculture have a long history of application, such as paper, board and various chemicals and materials. Over the last decades new bio-based products have emerged in the market. Some of the reasons for the increased interest lie in the bio-based products' benefits in relation to the depletion of fossil resources and climate change. Bio-based products may also provide additional product functionalities. This has triggered a wave of innovation with the development of knowledge and technologies allowing new transformation processes and product development.

Acknowledging the need for common standards for bio-based products, the European Commission issued Mandate M/492[1)], resulting in a series of standards developed by CEN/TC 411, with a focus on bio-based products other than food, feed and biomass for energy applications.

The standards of CEN/TC 411 "Bio-based products" provide a common basis on the following aspects:

— Common terminology

— Bio-based content determination

— Life Cycle Assessment (LCA)

— Sustainability aspects

— Declaration tools

It is important to understand what the term bio-based product covers and how it is being used. The term 'bio-based' means 'derived from biomass'. Bio-based products (bottles, insulation materials, wood and wood products, paper solvents, chemical intermediates, composite materials, etc.) are products which are wholly or partly derived from biomass. It is essential to characterize the amount of biomass contained in the product by, for instance, its bio-based content or bio-based carbon content.

The bio-based content of a product does not provide information on its environmental impact or sustainability, which may be assessed through LCA and sustainability criteria. In addition, transparent and unambiguous communication within bio-based value chains is facilitated by a harmonized framework for certification and declaration.

This European Standard has been developed with the aim to specify the method for the determination of oxygen content in bio-based products using an elemental analyser. This European Standard provides the reference test methods for laboratories, producers, suppliers and purchasers of bio-based product materials and products. It may be also useful for authorities and inspection organizations.

Part of the research leading to this document has been performed under the European Union Seventh Framework Programme OpenBio (see biobasedeconomy.eu)

[1)] A mandate is a standardization task embedded in European trade laws. Mandate M/492 is addressed to the European Standardization bodies, CEN, CENELEC and ETSI, for the development of horizontal European Standards for bio-based products.

1 Scope

This document provides an overview of existing applications of isotope ratio analysis of carbon, hydrogen, oxygen and nitrogen that are relevant to the analysis of bio-based feedstocks, products and production processes.

The stable isotope ratios of carbon, hydrogen, oxygen and nitrogen can be used to obtain information about the origin of bio-based feedstock and characteristics of production processes of bio-based products. However, no or limited attention for the use of the elements nitrogen and sulphur is given in this document due to the fact that these applications are not yet available.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 16575:2014, *Bio-based products - Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 16575:2014 apply.

4 Direct isotopic measurements

As described previously in Direct Automation OPEN BIO Deliverable N°3.7, isotopic measurements are performed using an Isotope Ratio Mass Spectrometer (IRMS). In order to test various samples (solids, liquids), automatic elemental analysers (EA)are connected to isotope ratio mass spectrometer for whole sample material for Bulk Stable Isotope Analysis (BSIA). This methodology is easy to operate, carries out fast analyses (few minutes), and has relative low cost enabling multi isotopic determinations. EA-IRMS is very suitable for regular authenticity controls of pure raw materials. Usually isotopic instruments are able to give the isotopic values of all organic elements contained in the samples.

Isotopic ratio mass spectrometer can also be connected to chromatography devices (separate methods) combustion or pyrolysis interface for Compound Specific Isotope Analysis (CSIA).Two different processes are available(GC-C/P-IRMS or LC-co-IRMS) depending on the molecules to be investigated. This approach is extremely appropriate for regular authenticity control of natural mixture samples (flavour, honey, fruit juice, essential oils…) and is well used in this scheme.

Isotopic composition is reported in Delta notation (δ) (in this case the isotopic composition of carbon is used as an example, other isotopes can be easily replaced in the formula):

$$\delta(^{13}C / ^{12}C) = \left[\frac{R(^{13}C / ^{12}C)sample}{R(^{13}C / ^{12}C)standard} - 1 \right] * 1000$$

The uncertainty of measurements carried out on modern devices in continuous flow isotope analysis is good and enough to make difference on the difference origins of targeted compounds.

The uncertainties for BSIA are close to these values (in delta notation) depending on the supplier:

δ13C: ± 0.3 ‰ / δ15N: ± 0.3 ‰ / δ2H: ± 5 ‰ / δ18O: ± 1‰.

Performances and precision of the different devices must be verified using references standards. International reference standards are supplied by different organisations (International Agency of Atomic Energy, National Bureau of Standards...) and validated by inter-comparison assessments.

5 Assessment of the authenticity of natural products

During the last decades the measurement of isotope ratios has acquired increasing importance in quality control, in the authenticity assessment of natural flavours, proof of authenticity of various food products. Although chemical methods can be used to detect contamination they are limited when looking at the geographical origin or to bring proof of authenticity. A high precision was developed for methods used to detect adulteration of natural products and particularly the addition of synthesis molecules. Methods based on the determination of $^{13}C/^{12}C$ ratios were first applied on molecules previously isolated and measured on offline combustion instruments.

The carbon isotopic composition of plants depends on the carbon dioxide assimilation and the fixation of carbon. Plants can be divided in three classes according to their metabolism assimilation. For most of the plants (so called C3 plants) the first intermediary molecule elaborated is the phosphoglycerate (molecule with 3 carbons atoms) and $\delta^{13}C$ generally range from −20 ‰ to −33 ‰. For the second class (C4 plants) the first intermediate molecule is a malate (molecule with 4 carbons) and $\delta^{13}C$ are generally in the range from −10 ‰ to −12 ‰ .Two plants are mainly representatives in this class: corn and sugar cane. Finally the third class concern plants which can process with the 2 pathway phosphoglycerate and malate (CAM plant) and the $\delta^{13}C$ ranges from −10 ‰ to −24 ‰. In this last category we can find vanilla and pineapple.

Among food flavours, vanilla has been probably the most investigated. Vanillin is the principal flavouring constituent of vanilla beans an orchid which operates according to the CAM pathway. $\delta^{13}C$ of vanillin origin beans is close to −20 ‰ where the $\delta^{13}C$ of synthesized vanillin are close to −28 ‰ when derived from wood lignin (C3 plant) and −29 ‰ when derived from guaiacol (J., 1982).

The development of a reference method about the detection of C4 plant sugars in honey by Jonathan White was a significant progress in the struggle of adulteration (WhiteJ.W., 1992). The methodology compares $\delta^{13}C$ of protein extracted from honey and used as internal standard with $\delta^{13}C$ of honey. A difference in excess of 1 permil in $\delta^{13}C$ evidences the presence of C4 additional sugar.

In 1990, the involvement of online coupling of high resolution gas chromatography (HRGC) with IRMS through combustion interface (HRGC-C-IRMS) has provided access to the analysis of individual constituents of complex flavouring products by measuring in particularly $^{13}C/^{12}C$ ratios (Gleixner, 1998). For food authenticity assessment, removal of the required extraction steps to isolate pure molecules was a significant time saving. Several applied methods based on GC-C-IRMS have been developed: Authenticity of essential oils such as Coriandrum (Franck C., 1995) mandarin oils (Faulhaber S., 1997) beverages such as whisky (ParkerI.G., 1998) oils such as olive oil (Angerosa F., 1997).

Plant water is always enriched in the heavy isotopes ^{18}O and ^{2}H related to the precipitation or groundwater, and this enrichment depends on plant transpiration as a function of assimilation type (C3, C4 or CAM plant). It can be assumed that the plant water ^{18}O enrichment relative to precipitation water decreases with increasing North latitude (Schmidt H.L., 2001).

The development of the online gas chromatography pyrolysis isotope ratio mass spectrometry (HRGC–P-IRMS) technique used for the quantification of the ratio $^{2}H/^{1}H$ allows to acquire new data in the authenticity of natural flavours assessment of natural origin of the main flavour compounds: Decanal, linalool, linalyl acetate (Hör K. R. C., 2001). All of these evidences results to the large variations in $^{2}H/^{1}H$ ratios in nature and the wide gap between natural and synthesis origins.

Furthermore the combination of ^{2}H and ^{13}C investigation using HRGC-C/P–IRMS has been a significant improvement in the knowledge of the assessment of natural molecules: citral (Hör K. R. C., 2001) (Trang T.T.N, 2006) ά and β ionone (Sewenig S., 2005) (Caja M.del M., 2007) ά and β decalactone (Tamura H., 2005).

The last step has been to associate the pyrolysis interface for the determination of $^{18}O/^{16}O$ isotope ratios in complement to the others isotope ($^{2}H/^{1}H$ and $^{13}C/^{12}C$) with the aim to deliver a three-dimensional plot. New applications were demonstrated for the authenticity of natural compounds: linalyl acetate and linalool in lavender essential oil (Jung J., 2005).

In 2004, the development of Liquid Chromatography coupled to stable carbon Isotope Ratio Mass Spectrometry via a Chemical Oxidation interface LC-Co-IRMS allowed new applications and perspectives in the authentication of origin. Twenty two amino acids were separated and the ^{13}C values determined. The results were similar to those extracted with chemical process and evaluated using an EA-IRMS approach (Godin J.P., 2005). Cabanero et al. (Cabanero A.I., 2010) showed a method allowing the determination of glycerol and ethanol. The results obtained were in good agreement with those performed using EA-IRMS. Guyon et al. (Guyon F., 2011) improved this method leading the determination of $\delta^{13}C$ of glucose, fructose, glycerol and ethanol in the same run for wine authentication check.

The development of laser spectroscopy permits to determine the organic isotope ratios in gases with an inherent compound–specific. Analytes do not necessarily need to be isolated, separated or trapped. Keppler et al. (KepplerF., 2010) showed the interest of the determination of ^{13}C in methane from anaerobic digesters. A large part of these isotopic methods are regularly undertaken to check the authenticity of flavours, essential oils and natural products in trading process. For industries involved in the fields of food, perfumes, essentials oils and cosmetics, stable isotopic approaches are definitely useful tools in the aim of validating the authenticity of natural products regarding synthesis adulteration, even if they know that the uncertainty could be quite important due to the variation of plants origins.

During the last decade, stable isotope laboratories involved in authenticity assessment collected lots of isotopic data related to the different origins of molecules. This work is important and must be continued to ensure a steady evaluation of the database collected for authenticity validation.

Some isotopic methods have been also recognized as international methods. The list in Table 1 gives some examples of the official methods elaborated in the naturalness authenticity assessment.

Table 1 — Examples of official methods using stable isotope ratios

Organization	Method reference	Title
CEN	ENV 12140	Fruit and vegetables juices - Determination of the stable carbon isotope ratio (13C/12C) of sugars from fruit juices-method using isotope ratio mass spectrometry
CEN	ENV 12141	Method for determination of stable oxygen isotope ratio (18O/16O) of water from fruit juices, using isotope ratio mass spectrometry
CEN	ENV 12142	Method for determination of stable hydrogen isotope ratio (2H/1H) of water from fruit juices, using isotope ratio mass spectrometry
AOAC[1]	Method 998.12	Detection of C-4 Plant Sugars in Honey by 13C/12C analysis
AOAC	Methods 981.09 and 982.21	Detection of addition of beet sugars in fruit juices (13C/12C analysis)
AOAC	Method 984.23	Carbon isotope ratio mass spectrometric method for detection of corn syrup and cane sugar in maple syrup
AOAC	Method 992.09	Determination of sugar beet derived syrups in frozen concentrated orange juice- δ18O measurements in water
AOAC	Method 2004.01	Carbon stable isotope ratio of ethanol derived from fruit juices and maple syrups

Organization	Method reference	Title
OIV[2]	Resolution OENO/7/2001	Determination by isotope ratio mass spectrometry of 13C/12C of wine ethanol or that obtained through the fermentation of musts, concentrated must or grape sugar
OIV	Resolution OENO/7/2005	Determination of the carbon isotope ratio 13C/12C of CO_2 in sparkling wines method using isotope ratio mass spectrometry (IRMS)
[1] Association of analytical communities [2] International Organization of vine and wine		

Isotopic analysis used for the assessment of natural product are undertaken to validate (or not) the authenticity of the target samples. In the field of biobased product, a compound could be biobased, non-biobased or partly biobased with a known level of biobased content. If the isotopic methodology would be used as an accepted method, it could bring this assessment of the biobased content with the higher acceptable uncertainty.

6 Overview of feedstock isotopic fingerprint

6.1 C4 plant

Sugar cane and maize have a specific isotopic ^{13}C fingerprint due to their belonging to the C4 photosynthesis pathway cycle and are among the major feedstock employed.

Sugar cane

Rodushkin et al. (Rodushkin I., 2011) present the results of an inter-laboratory program based on the multi elements and isotopic measurements of several sugar samples with different geographical origins: USA, Costa Rica, Argentina and Swaziland. The results obtained on these cane sugar samples were:

$\delta^{13}C$ Values range from −10.5 to −12.6 ‰ .

$\delta^{13}C$ Average −11.70 ‰ SD 0.52 ‰

Two samples have been analysed (see Table 2).

Table 2 - measurement of sugar cane samples from various geographical origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
sugar	cane	Paraguay	−11.71	−3	35
		Reunion Island	−11.66	11	37

Corn

Several samples of starch have been analysed. They originate from the fields of production feeding the biobased industry factories (Table 3).

Table 3 - Measurements of corn samples from various geographical origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
starch	corn	China	−11.66	−28	30
		France	−11.44	−16	32
		Italia	−11.81	−19	32
		Spain	−11.49	−21	32
		France	−11.42	−2.5	33
		Brazil	−10.72	−21	29
		Brazil	−11.02	−25	29
		USA	−10.82	−17	29
		Turkey	−11.51	−22	31

$\delta^{13}C$ Values range from −11.81 to −10.72 ‰ .

$\delta^{13}C$ Average −11.34 ‰ SD 0.35 ‰

$\delta^{13}C$ measured on C4 plant origin sample were in good agreement with data published.

6.2 Other raw materials: C3 plants

Rodushkin et al. (Rodushkin I., 2011) have reported the results of an inter-laboratory program based on the multi elements and isotopic measurements of several sugar samples with different geographical origins: Moldavia, Poland, France, Netherlands, Germany, Hungary and USA. The results obtained on various cane sugar samples were:

$\delta^{13}C$ Values range from −23.8 to −26.5 ‰ .

$\delta^{13}C$ Average −24.98 ‰ SD 0.75 ‰

Starch which is a significant raw-material could be originated from various origins. Table 4 presents the $\delta^{13}C$ of starch samples from C3 plants.

Table 4 - Measurements of starches from various origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
	wheat	France	−26.91	−47	31
		Corby	−27.00	−43	32
		France	−27.21	−43	32
	pea	France	−27.38	−40.5	33
	potatoes	Denmark	−28.44	−102	27
		France	−26.51	−79	35
	tapioca	Brazil	−26.11	−82	29

$\delta^{13}C$ measured of C3 starch samples range from −26 to −28 ‰. $\delta^{18}O$ obtained on C3 and C4 starch plant origins seem to be in the same isotopic area.

7 Determination of Biobased content for feedstocks and products

7.1 Bioplastics

Current plastics products are composed of biobased synthetic polymers, fossil-based synthetic polymers, natural polymers and additives that can include biobased materials. “Biobased plastic” refers to plastic that contains materials wholly or partly of biogenic origin (Plastics - Biobased Content Part 5: declaration of biobased carbon content, biobased synthetic polymer content and biobased mass content).

Polyethylene Terephthalate (PET)

In 2009 the Coca-Cola Company presented their Plant Bottle® PET packaging innovation made from up to 30 % biobased, sugar cane renewable materials. The new product encompasses a part of MEG (mono ethylene glycol) biobased origin in the PET final product.

In response, the Coca-Cola Analytical Science Team has developed a novel patent (pending) using an analytical method to quantify the amount of biobased material in Plant Bottle® PET resin. This new approach has shown good reproducibility and accuracy, with excellent correlation to the conventional ASTM 6866 method using radiocarbon analysis. The new method is performed using an elemental analyser TOC (total organic carbon) connected to a cavity ring-down spectroscopy (CRDS) detector (TOC-CRDS). This equipment allows the determination of the delta ^{13}C value from a sample after combustion. The correlation between the delta ^{13}C values and the ^{14}C measurements connected to the biobased carbon content were in good accordance allowing the ^{13}C method to be an efficient alternative method in this particular industrial process. Rapidity and relatively inexpensive test are also significant advantages of the alternative stable isotopic approach (Brevet n° WO 2012/174104 A1, 2012).

Suzuki et al. presented the ability of $\delta^{13}C$ method to discriminate between plant and petroleum derived plastics (Suzuki Y., 2010). The δ ^{13}C values of the plastics investigated range from −17.3 ‰ to −10.0 ‰ for corn derived plastics PLA (Poly-Lactic acid), from −28.6 ‰ to −25.8 ‰ for sugar cane-derived plastics PE (polyethylene) from −28.6 ‰ to −25.8 ‰ for rice-derived plastics PLA and from −32.1 ‰ to −25.4 ‰ for petroleum derived plastics PE. The $\delta^{13}C$ results obtained suggest that plastics derived from C4 plants are clearly significant higher than the fossil origins.

In addition, several PET mineral water bottles have been collected from the French market for a multi-isotopic approach. PET is made by an esterification reaction between terephthalic acid and ethylene glycol. Only ethylene glycol was biobased since it was made from cane sugar. The plastic elaborated has 31 % of biobased content, and if recycled PET is added (35 %), this biobased content part decreases to 20 %.

Sample preparation has been directly carried out by cutting out small parts of the plastic bottles. Determination was done according to EA-IRMS method.

The results in Table 5 present different types of origins of the samples:

— Sample M1 and M2 called “green bottle” are partly biobased and the % of biobased content is the statement given by the manufacturer.

— Samples from M3 to M6 are fossil originated bottles