SLOVENSKI STANDARD
oSIST prEN 18054:2024

01-april-2024

Pristnost živil - Določanje razmerja izotopov C in/ali N v živilih z elementnim analizatorjem - masno spektrometrijo izotopskega razmerja (EA-IRMS)

Food authenticity - Determination of C and/or N isotope ratios in food by Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS)

Lebensmittelauthentizität - Bestimmung von C- und/oder N-Isotopenverhältnissen in Lebensmitteln mittels Elementaranalyse mit Isotopenverhältnis-Massenspektrometrie (EA□IRMS)

Authenticité des aliments - Détermination des rapports isotopiques du carbone et/ou de l’azote dans les aliments au moyen d’un analyseur élémentaire couplé à un spectromètre de masse de rapports isotopiques (AE-SMRI)

Ta slovenski standard je istoveten z: **prEN 18054**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

oSIST prEN 18054:2024 **en,fr,de**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

DRAFT
prEN 18054

February 2024

ICS 67.050

English Version

Food authenticity - Determination of C and/or N isotope ratios in food by Elemental Analyser - Isotope Ratio Mass Spectrometry (EA- IRMS)

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 460.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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European foreword

This document (prEN 18054:2024) has been prepared by Technical Committee CEN/TC 460 “Food Authenticity”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document provides the base for the analytical methods. The setup of the required apparatus depends to a large extent on its design principles and the specific recommendations of the manufacturers have to be followed. It is intended to serve as a frame in which the analyst can define his own analytical work in accordance with the standard procedure.

Introduction

Stable isotope ratios of carbon and nitrogen are often expressed as isotope delta values (δ) these are ratios of the heavier to the lighter isotope of the element in a particular sample relative to the same ratio within an agreed reference.

Carbon stable isotope ratios can provide information regarding the photosynthetic pathway that forms the base of the food chain for the biological material in question. For example, the difference between the Calvin-Benson cycle used by C_3 plants and the Hatch-Slack pathway used by C_4 plants during photosynthesis results in a measurable difference in the ratio of ^{13}C to ^{12}C for the sugars and other subsequent molecules in biosynthesis.

Nitrogen stable isotope ratios also vary between food groups. The ratio of ^{15}N to ^{14}N increases with trophic level due to preferential excretion of ^{14}N containing molecules such as urea or uric acid. Nitrogen isotope ratios in plant material are also affected by the fertilization regime.

Analysis of stable isotope ratios within foodstuffs has the potential to reveal information regarding origin, both biological and geographical, adulteration and authenticity. For example, adulteration of honey from C_3 plants with refined sugars of C_4 origin can be detected, as can the difference between maize (corn, a C_4 plant) and wheat or other grain-fed chicken.

This document concerns instrumental methods for determination of carbon and/or nitrogen isotope delta values in foodstuffs.

1 Scope

This document covers instrumental analysis by elemental analyser-isotope ratio mass spectrometry (EA-IRMS) of food materials to determine C and/or N isotope ratios.

The isotope ratios obtained by following this document are expressed as δ^{13}C and/or δ^{15}N values relative to international measurement standards.

Sample preparation is not included within this document. It is assumed that the food sample has been pre-treated as necessary and homogenized.

Similarly, the interpretation of the obtained isotope delta values is not covered by this document. Following this protocol will result only in isotope delta values for the sample materials.

Solid and/or liquid sample materials can be analysed following this document.

Although other instrumental techniques can be applied to determine δ^{13}C and/or δ^{15}N values in food materials, these other techniques are not covered by this document.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp/
- IEC Electropedia: available at https://www.electropedia.org/

3.1
isotope delta

δ

stable isotope ratio of a material expressed relative to a reference according to the example expression using carbon:

$$\delta_{\text{ref}}\left({}^{13}\text{C}/{}^{12}\text{C}\right)=\frac{R_{\text{sample}}\left({}^{13}\text{C}/{}^{12}\text{C}\right)}{R_{\text{ref}}\left({}^{13}\text{C}/{}^{12}\text{C}\right)}-1 \quad (1)$$

Note 1 to entry: *ref* is the reference.

Note 2 to entry: A similar expression can be constructed for nitrogen isotope delta using ^{15}N/^{14}N in place of ^{13}C/^{12}C.

Note 3 to entry: The terms δ_{ref}(^{13}C/^{12}C) and δ_{ref}(^{15}N/^{14}N) are often changed from the correct IUPAC format to $\delta\,^{13}\text{C}_{\text{ref}}$ and $\delta\,^{15}\text{N}_{\text{ref}}$. This document uses the latter expressions for familiarity.

Note 4 to entry: To ensure international comparability of isotope delta values, a common reference is used for each isotope ratio. This reference is an international measurement standard assigned by convention with isotope delta value exactly equal to zero.

Note 5 to entry: Isotope delta values reported against the same common reference are said to be on the same scale. The name of the scale is usually the same as that of the reference.

Note 6 to entry: Carbon and nitrogen isotope delta values for natural isotopic abundance food materials are small and expressed in permille (‰) rather than in their native form.

3.2
Vienna peedee belemnite
VPDB

international measurement standard for δ^{13}C

Note 1 to entry: VPDB is a virtual carbonate.

Note 2 to entry: The carbon isotopic composition of VPDB is defined by the exact isotope delta value assigned to the calcium carbonate reference material NBS 19 of $\delta^{13}C_{VPDB} = +1,95$ ‰.

Note 3 to entry: To ensure traceability to the VPDB scale, carbon isotope delta values are normalized using two or more reference materials to account for scale effects during measurement (7.6.4.5) [1].

3.3
atmospheric nitrogen
Air-N_2

international measurement standard for δ^{15}N

Note 1 to entry: The Air-N_2 nitrogen isotope delta scale is defined by the exact isotope delta values assigned to atmospheric nitrogen and the potassium nitrate reference material USGS32 of $\delta^{15}N_{Air\text{-}N2} = 0$ and +180 ‰, respectively [1,2].

Note 2 to entry: To ensure traceability to the Air-N_2 scale, nitrogen isotope delta values are normalized using two or more reference materials to account for scale effects during measurement (7.6.4.5) [1].

3.4
sample gas

analyte gas obtained by conversion of the sample material within the elemental analyser

Note 1 to entry: The sample gas is carbon dioxide (CO_2) for δ^{13}C determination and nitrogen gas (N_2) for δ^{15}N determination.

3.5
working gas

consisting of the same molecule as the analyte gas (i.e. CO_2 or N_2) but introduced directly into the mass spectrometer from a high-pressure cylinder rather than being created from a solid/liquid sample material within the elemental analyser

Note 1 to entry: Also referred to as “cylinder gas” or “monitoring gas” or, incorrectly, “reference gas”.

Note 2 to entry: An isotope delta value with a working gas within its traceability chain to the international measurement standard will require normalization using reference materials of known isotope delta analysed in the same sequence before the isotope delta value lies on the international scale.

3.6
sequence

continuous set of analyses including reference materials for normalisation, QA/QC materials, procedural blanks and samples prepared and analysed together

Note 1 to entry: Also referred to as “batch”.

4 Principle

4.1 General

A sector field mass analyser (Isotope Ratio Mass Spectrometer, IRMS) allows measurement of isotope ratios with very high precision in simple gases.

Prior determining the carbon and nitrogen isotope signature of solid or liquid samples they shall be converted into CO_2 and/or N_2 gas.

This happens in an elemental analyser (EA) via high temperature combustion and subsequent conversion, cleaning and separation steps.

4.2 Sample introduction

Homogenized sample material should be weighed into tin capsules, boats or foil, sealed and then loaded into the carousel of an autosampler. Autosamplers should allow contamination-free introduction of the capsules into the combustion reactor. Liquid samples up to a certain viscosity can be injected by liquid autosampler via airtight septa. Those of higher viscosity can be weighed into smooth walled tin capsules and sealed with appropriate devices.

4.3 Flash-combustion and reduction

4.3.1 General

In the EA the sample material is quantitatively oxidized to avoid isotope fractionation. This happens in a reactor made of quartz or steel, which is continually flushed with high purity He carrier gas. The reactor temperature is typically maintained between 900 °C to 1 200 °C according to the manufacturer's specifications.

To ensure full sample combustion, O_2 gas is injected into the helium stream ("O_2 pulse"). The O_2 shall be in stoichiometric excess to archive full oxidation of the sample. However, the unused O_2 shall be eliminated by reduction to prevent O_2 from entering the separation system, and/or the IRMS.

Full oxidation is facilitated by an oxidation catalyst. The reactor typically contains [copper (II), chromium (III) or W(VI) oxides] and a scavenger to bind sulphur and halogens [cobalt (II, III) oxide and/or silver], although many variations are recommended by manufacturers for specific applications.

The heat of the reaction is supported by the oxidation of the tin capsules, boats or foils which increases the temperature to about 1800 °C (flash burning).

Combustion results in the following gases: N_2, NO_x, CO_2 and H_2O. Other by-products such as halogens and sulfur oxides are eliminated with a scavenger material as mentioned before.

Incombustible materials remain in the reactor in the form of ash.

The reduction of the NO_x to N_2 takes place at lower temperatures, either in a cooler part of a single tube or in a separate furnace, typically maintained at between 600 °C and 850 °C. Excess oxygen will also be removed in this way. The reduction process typically relies on high purity elemental copper or tungsten and, again, variations are recommended for specific applications.

Following the combustion/reduction reactor(s) there should be a water trap to remove the moisture evolved during combustion.

4.3.2 Inorganic nitrogen

Traditional combustion methods, used to produce N_2 for isotopic measurements, are not quantitative for materials containing nitrogen in high oxidation states, specifically nitrates [3,4].

This can lead to bias in nitrogen isotope ratio results and conversion via thermal decomposition as opposed to combustion is recommended.

The EA configuration for analysis of nitrates is the same as for combustion except it is recommended that the "O_2 pulse" is disabled and the method timing is changed slightly such that samples decompose at high temperature rather than combust.

The net effect is a decrease in the amount of NO_x versus N_2 produced during thermal conversion of samples to gas.

4.4 Gas separation

The N_2 and CO_2 gases are separated by either a gas chromatography (GC) column or using a "purge-and-trap" system to achieve separation [5].

With purge-and-trap nitrogen passes directly through the system while other evolved gases (CO_2, etc.) are collected on one or more adsorption tubes (effectively short GC columns). These traps are then sequentially heated to liberate the gases into the MS.

When only nitrogen isotope ratios are to be determined, CO_2 can be removed from the gas stream using a chemical trap containing soda lime or sodium hydroxide on a silica substrate. These reagents produce water when absorbing CO_2 and should be positioned between two water traps.

The sample gases, in particular CO_2, may be diluted by an additional flow of helium gas within the carrier flow prior to transfer to the IRMS.

4.5 Mass spectrometer measurement

The gas molecules are ionized in an electron impact (EI) ion source and the major isotopologue ions of the molecular ion are measured.

After acceleration by a static electric field, ions are separated by momentum. The separator is usually an electromagnet, although permanent magnets have also been used.

The ions are detected in a set of detectors (mostly Faraday cups), which simultaneously collect the ions of interest.

NOTE The Faraday cups collect ions with m/z values of 44, 45 and 46 when measuring carbon isotope ratios of CO_2 and ions with m/z values of 28, 29 and 30 when measuring nitrogen isotope ratios of N_2.

Typically, the IRMS instrument software automatically calculates raw isotope-delta values that can be used for subsequent data processing. This process will involve the integration of the sample and working gas peak signals from the Faraday collectors; calculation of ratios of these integrated ion currents, correction for isobaric interferences where necessary (e.g. correction for the contribution of 12C17O16O to the m/z 45 signal) and conversion of the corrected ratios to raw isotope delta values. The user may need to specify various parameters such as known/assigned isotope delta value(s) of the working gas.

4.6 Corrections to initial isotope delta values

Several effects may be present within initial isotope delta values. These include blank contributions; drift in isotope delta with time within a sequence; a mass (or linearity effect) whereby the isotope delta value obtained for a material depends on the amount of the element of interest analysed; or memory/carryover effects.

The need to apply these corrections should be determined by including procedural blanks and quality control materials within each analytical sequence. Further details regarding these corrections can be found in 7.6.4.