SLOVENSKI STANDARD
oSIST prEN 17958:2023

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Avtentičnost hrane - Določanje vrednosti d13C mono- (fruktoza in glukoza), di- in trisaharidov v medu z masno spektrometrijo, tekočinsko kromatografijo in izotopskim razmerjem (LC-IRMS)

Food authenticity - Determination of the d13C value of mono- (fructose and glucose), di-, and trisaccharides in honey by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS)

Lebensmittelauthentizität - Bestimmung des δ13C-Wertes von Mono- (Fructose und Glucose), Di- und Trisacchariden in Honig durch Flüssigchromatographie-Isotopenverhältnis-Massenspektrometrie (LC-IRMS)

Authenticité des aliments - Détermination de la valeur du δ13C des mono- (fructose et glucose), di-, et trisaccharides présents dans le miel par chromatographie en phase liquide spectrométrie de masse de rapports isotopiques (CL-SMRI)

Ta slovenski standard je istoveten z: **prEN 17958**

ICS:

67.180.10 Sladkor in sladkorni izdelki Sugar and sugar products

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Food authenticity - Determination of the $\delta^{13}C$ value of mono- (fructose and glucose), di-, and trisaccharides in honey by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS)

Lebensmittelauthentizität - Bestimmung des $\delta^{13}C$-Wertes von Mono- (Fructose und Glucose), Di- und Trisacchariden in Honig durch Flüssigchromatographie-Isotopenverhältnis-Massenspektrometrie (LC-IRMS)

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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 17958:2023) 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.

Introduction

Honey is a natural sweetener having attractive sensory properties. Demand has increased over the years, partially due to population increase, but also due to the preference of consumers for natural and unprocessed food. It is a globally traded commodity following complex trade routes, which makes quality and authenticity control difficult. Honey is among the commodities most vulnerable to fraud [1]. The EU Honey Directive lays down the composition and authenticity criteria for honey [2]. A set of analytical methods have been standardized by the International Honey Commission [3], which allows enforcement of the provisions of the Directive. Those methods are widely used but are not always appropriate for assessing the authenticity of the product: specifically, adulteration of honey by non-declared dilution with foreign sugars/sweeteners, which is among the most frequently encountered cases.

Syrups that mimic the composition of honey that are produced by chemical and/or enzymatic modification of starch or sucrose are difficult to detect [4]. If the starting product is obtained from a plant using the Hatch–Slack pathway for carbon fixation (C4 plant), such as maize or sugar cane, stable carbon isotope ratio analysis (SCIRA) using a combination of an elemental analyzer and an isotope ratio mass spectrometer (EA-IRMS) offers a possibility to detect additions down to a level of 7 % [5]. Sugars originating from C3 plants, which use the Calvin–Benson cycle, such as beet root or sugars generated from rice or wheat starch escape detection by SCIRA. Combining liquid chromatography (LC) with IRMS (LC-IRMS) offers new possibilities for detecting honey adulteration with sugars derived from C3 plants as well as increasing the sensitivity for detecting C4 sugars [6][7]. The method has gained popularity but has never been subjected to multi-laboratory validation, which is a prerequisite for further developing it into a standard by a Standards Developing Organization.

An LC-IRMS method for the determination of the $^{13}C/^{12}C$ isotope ratios of glucose, fructose, glycerol and ethanol in products of viti-vinicultural origin was collaboratively studied by the International Organization of Vine and Wine (OIV) and endorsed for inclusion in the Compendium of International Methods of Analysis of Wines and Musts (OIV-OENO resolution 479-2017).

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

1 Scope

This document specifies a method for the determination of the ratio of stable isotopes of carbon ($^{13}C/^{12}C$) of sugars contained in honey by using liquid chromatography coupled to an isotope ratio mass spectrometer (LC-IRMS) for compound separation and subsequent determination of the $^{13}C/^{12}C$ ratio of mono-, di-, and trisaccharides. These ratios can be used to assess honey authenticity by comparing them to published guidance values of genuine honey as the $^{13}C/^{12}C$ ratios of sugars of genuine honey and sugars contained in adulterants (syrups made from starch-rich plants or from sugar cane or sugar beet) differ to a certain extent. The compliance assessment process is not part of 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:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
isotope delta
δ
stable isotope ratio of a sample expressed relative to a reference

Note 1 to entry: For carbon, this expression is given in Formula (1):

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

Note 2 to entry: The term $\delta_{\text{ref}}(^{13}C/^{12}C)$ is often changed from the IUPAC format to $\delta^{13}C_{\text{ref}}$; this document uses the IUPAC format for familiarity.

Note 3 to entry: To ensure international comparability of isotope delta values, a common reference is used; this reference is an international measurement standard assigned by convention with isotope delta value exactly equal to zero.

Note 4 to entry: Carbon isotope delta values for natural isotopic abundance in food materials are small and expressed in permille (‰) rather than in their native form.

3.2
Vienna Peedee Belemnite
VPDB
international measurement standard for $\delta^{13}C$

Note 1 to entry: $\delta^{13}C$ is a virtual carbonate.

3.2.1
VPDB carbon isotope delta scale
exact isotope delta value assigned to the calcium carbonate reference material NBS 19 of $\delta^{13}C_{VPDB}$ = +1,95 ‰

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

3.3
mass-to-charge ratio
m/z
dimensionless quantity formed by dividing the mass number of an ion by its charge number

3.4
working gas
gas consisting of the same molecule as the analyte gas (i.e. CO_2) but introduced directly into the mass spectrometer from a high-pressure cylinder rather than being created from the sample

3.5
sequence
continuous set of analyses including reference materials for normalization, QA/QC materials, procedural blanks and samples prepared and analyzed together

4 Principle

Honey is diluted with water, filtered and injected into a liquid chromatography system for separation into mono-, di-, tri- and oligosaccharides. The column effluent is fed into an interface where organic compounds are oxidized to CO_2. CO_2 isotopologues with *m/z* 44, 45 and 46 are separated in the spectrometer and detected using Faraday cups. Compound specific $\delta^{13}C$ (‰) values are then calculated according to Formula (1).

5 Reagents

Unless otherwise stated, use only reagents of recognized analytical grade (purity ≥ 99 %) and ultrapure water with a resistivity > 18 MΩ cm^{-1}.

5.1 Working gas, carbon dioxide, 99,995 %.

5.2 Helium, 99,999 %.

5.3 Certified reference materials, at least two reference materials with certified $\delta^{13}C$ values traceable to VPDB and spanning the expected measurement range shall be used for calibration[1]. When used for calibration, the $\delta^{13}C$ values and the associated uncertainties stated on the accompanying certificates shall be used. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the products. Aqueous solution of those reference materials shall be prepared and used for calibration according to the specifications of the instrument vendor.

[1] Benzoic acid (IAEA-601), Glucose (BCR-657), Beet sugar (BEET-1), Fructose (FRUT-1), Galactose (GALT-1), Sucrose (UME CRM 1309), Glucose (UME CRM 1310), Fructose (UME CRM 1311), Honey (Unadulterated) (UME CRM 1312), Honey (Adulterated) (UME CRM 1313) are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

5.4 Reference materials, sugars such as fructose, glucose, or sucrose of analytical grade (purity > 98 %) calibrated against certified reference materials traceable to VPDB, can be used to normalize measured isotope δ values to the VPDB scale and to check for drift during operation of the isotope ratio mass spectrometer. Honey which has the $\delta^{13}C$ values of its main sugars characterized by an appropriate proficiency testing (PT) scheme can be used for quality control.

5.5 Sodium peroxodisulfate or ammonium peroxodisulfate.

5.6 Ortho-Phosphoric acid.

NOTE 5.5 and 5.6 are required in case a chemical oxidation interface (6.4) is used.

6 Apparatus

Usual laboratory equipment, and in particular, the following:

6.1 Liquid-chromatography (LC) system, consisting of a pump, mobile phase degasser, column heater and sample injection device.

6.2 LC column, suitable of separating mono-, di-, tri-, and oligo-saccharides using only water as mobile phase shall be used. Polymeric styrene-divinylbenzene resins loaded with Ca^{2+} and a particle size of 8 µm to 10 µm are suitable stationary phases. Appropriate column dimensions are 300 mm in length and between 6 mm and 8 mm in diameter; the typical flow-rate of the mobile phase (high purity water, 6.3) for such columns is between 0,2 ml/min and 0,6 ml/min, depending on the column type used.

The column shall be operated at a temperature between 50 °C and 70 °C.

NOTE Column types, dimensions, and flow-rates used in the collaborative study are given for informational purposes in Annex A; other column types and particle sizes are also used.

6.3 LC mobile phase, water, HPLC grade (resistivity > 18 MΩ cm^{-1}).

6.4 Interface, for online coupling the LC system to the isotope ratio mass spectrometer and oxidising carbon containing compounds to CO_2 either by chemical oxidation or combustion.

6.5 Isotope ratio mass spectrometer (IRMS), with a working gas inlet and collectors registering CO_2 isotopologues with *m/z* 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$).

NOTE The ion current *m/z* 45 is corrected for the contribution of $^{12}C^{17}O^{16}O$, which is computed from the current intensity measured for *m/z* 46 by considering the relative abundance of ^{18}O and ^{17}O.

6.6 Data system, controlling the LC-system, the LC-IRMS interface, the IRMS, data acquisition and calculation of $\delta^{13}C$ values.

6.7 Filtration devices, to filter the LC mobile phase (water) and sample solutions.

NOTE A pore size of 0,45 µm is appropriate for filtering sample solutions.

6.8 Water bath or oven, at 40 °C ± 2 °C.

6.9 Laboratory balance, with an accuracy of ± 0,01 g.

6.10 Volumetric flask, 20 ml.

7 Procedure

7.1 Preparation of the test sample

Warm the honey sample at 40 °C ± 2 °C for approximately 30 min in a closed container, open the container, stir the content with a glass rod.

7.2 Preparation of the test sample solution

Transfer 0,1 g sample into a 20 ml volumetric flask (6.10) and make up to the mark with water. Filter through a 0,45 µm filter (6.7) before the determination.

NOTE Other sample concentrations are used, depending on the LC-IRMS system set up used.

7.3 LC-IRMS setup and performance qualification

7.3.1 LC-IRMS interface setup

Set up and test the LC-IRMS system according to the manufacturer's instructions.

In case carbon containing compounds are chemically oxidized to CO_2 in the interface, prepare aqueous solutions of sodium peroxodisulfate (5.5) and phosphoric acid (5.6), according to the manufacturer's instructions.

7.3.2 IRMS calibration

Use at least two certified reference materials (5.3) having compound specific $\delta^{13}C$ values in the range of approximately −10 ‰ to −30 ‰ to calibrate the IRMS system, which is used to determine the compound specific $\delta^{13}C$ values of saccharides in each analytical run, following the manufacturer's instructions.

7.4 Determination and analytical sequence

Set up an analytical sequence which includes test samples, procedural blanks, QC/QA samples and an appropriate number of reference samples with known compound specific $\delta^{13}C$ values.

Inject an appropriate volume of the sample solutions (7.2), usually 10 µl to 20 µl, and program the LC-IRMS system to introduce three working gas (5.1) pulses of about 20 s each at the beginning of each analytical run.

7.5 Calculations

7.5.1 General

The calculation algorithms of the data system (6.6) can be used, provided equivalent results are obtained.

7.5.2 Calculation of compound specific $\delta^{13}C$ values using two-point linear normalization

The $\delta^{13}C$ value relative to VPDB [in ‰] of an individual compound is calculated according to Formula (2):