Food authenticity - Determination of the δ13C value of mono- (fructose and glucose), di-, and trisaccharides in honey by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS)

EVS EESTI STANDARDIMIS- JA AKREDITEERIMISKESKUS
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EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 17958:2024 sisaldab Euroopa standardi EN 17958:2024 ingliskeelset teksti.	This Estonian standard EVS-EN 17958:2024 consists of the English text of the European standard EN 17958:2024.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 27.11.2024.	Date of Availability of the European standard is 27.11.2024.
Standard on kättesaadav Eesti Standardimis-ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 67.180.10, 67.180.20

EUROPEAN STANDARD

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English Version

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)

Authenticité des aliments - Détermination de la valeur du $\delta^{13}C$ 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)

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

This European Standard was approved by CEN on 24 June 2024.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

This document (EN 17958:2024) has been prepared by Technical Committee CEN/TC 460 “Food Authenticity”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

Honey is a natural sweet substance produced by honey bees 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 harmonized 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 analyser 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 delta values 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 guidance values of genuine honey, which have been previously agreed by subject matter experts, 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: VPDB is a virtual carbonate.