



SLOVENSKI STANDARD

oSIST prEN 17992:2023

01-september-2023

Pristnost živil - Določanje vsote 16-O-metilkafeola, 16-O-metilkafeola in njihovih derivatov v praženi kavi z metodo ^1H -qNMR

Food authenticity - Determination of the sum of 16-O-methylcafestol, 16-O-Methylkahweol and their derivatives in roasted coffee by ^1H -qNMR

Lebensmittelauthentizität - Bestimmung des Gehalts von 16-O-Methylcafestol, 16-O-Methylkahweol und deren Derivaten als Summenparameter in Röstkaffee mittels ^1H -qNMR

Authenticité alimentaire du café - Détermination de la teneur en 16-O-méthylcafestol - Méthode par NMR

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English Version

Food authenticity - Determination of the sum of 16-O-Methylcafestol, 16-O-Methylkahweol and their derivatives in roasted coffee by ^1H -qNMR

Authenticité alimentaire du café - Détermination de la teneur en 16-O-méthylcafestol - Méthode par NMR

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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 17992:2023) 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.

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Introduction

This document was developed in response to demand for an efficient and reliable test method allowing the confirmation of coffee authenticity both for commercial quality control and for official food control.

The coffee species with the greatest commercial importance are *Coffea Arabica* and *Coffea canephora var. robusta*, commonly known as “arabica” and “robusta”. Within these species, Arabica coffees have a significantly higher market value than robusta coffees. In unblended coffee the botanical origin of coffee can be determined unambiguously by analysing the concentration of 16-O-methylcafestol and 16-O-Methylkahweol and their derivatives (e.g. fatty acid esters) because these marker substances are present in arabica coffee at very low concentrations (typically < 20 mg/kg). In robusta coffees the concentration levels are significantly higher in the approximate range of 800 to 2 500 mg/kg.¹ [1 – 5]

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¹ These published values are quoted as an orientation only and not intended as strict threshold recommendations.

1 Scope

This document specifies a method for the determination of soluble 16-O-Methylcafestol and 16-O-Methylkahweol content (the sum of free forms and derivatives, e.g. fatty acid esters, henceforth abbreviated as 16-OMD = “diterpenes”) in roasted coffee (beans or ground), using quantitative proton nuclear magnetic resonance spectroscopy (^1H -qNMR).

If complying with the experimental parameters described below, this test procedure has been proven for the following concentration range:

16-OMD: 20 mg/kg to 2 000 mg/kg.

The concentration range can be expanded by suitable changes of the experimental parameters, e.g. a different weighed portion of ground coffee or the accumulation of more NMR-transients.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 1042, *Laboratory glassware - One-mark volumetric flasks (ISO 1042)*

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

ISO 3509, *Coffee and coffee products — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 3509 and the following 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

16-OMC and 16-OMK (= 16-O-Methyl Diterpene (“16-OMD”) derivatives content extractable with chloroform

content in coffee, determined according to this test procedure and quoted as a mass fraction w in mg per kg coffee, calculated as free 16-OMC

Note 1 to entry: An additional fraction of the total 16-OMD content (ca. 25 %) can only be extracted by vigorous chemical processing.

Note 2 to entry: Approximately a mass fraction of 90 % of the 16-OMD is represented by 16-O-methylcafestol, and 16-O-methylkahweol represent the remaining mass fraction of 10 % of the mixture.

Note 3 to entry: The relative error due to differences in molar weight is 0,6 %.

[SOURCE: [1]]

prEN 17992:2023 (E)

3.2

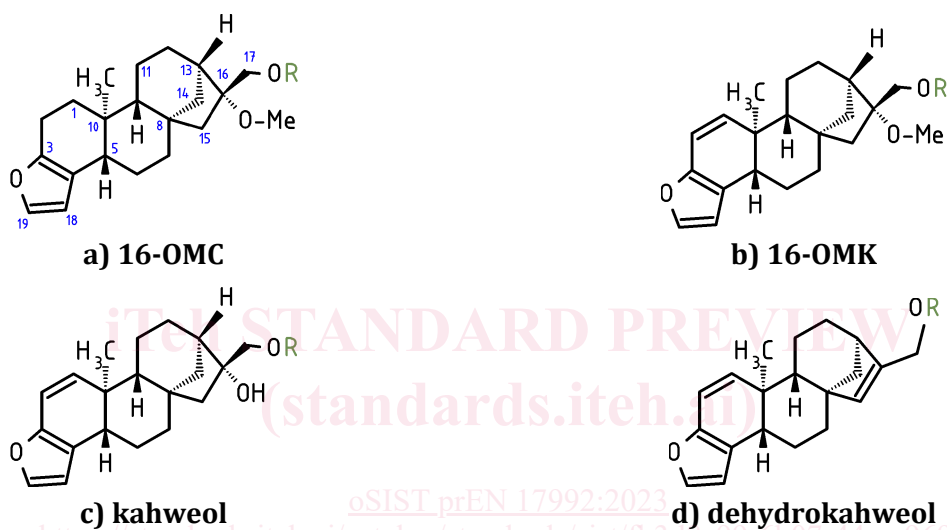
16-OMC**16-O-Methylcafestol**(CAS RN^{®2}: 108214-28-4), $M = 330,5$ g/mol

Note 1 to entry: The substance appears in its free form or esterified, see Figure 1.

3.3

16-OMK**16-O-Methylkahweol**(CAS RN[®]: not yet available), $M = 328,5$ g/mol

Note 1 to entry: The substance appears in its free form or esterified, see Figure 1.

**Key**

R residue (H or fatty acid)

NOTE The atom numbering is the same for the shown substances.

Figure 1 — Four diterpenes present in coffee

3.4

FID**free induction decay**

time-domain NMR signal that results from the precession of the nuclear magnetization vector inside the probe coil after application of an excitation RF pulse to a sample in a static magnetic field

[SOURCE: ISO 24583:2022, 3.6]

² CAS Registry Number[®] is the trademark of a product supplied by CAS. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3.5**FWHM****full width at half maximum**

width of a line shape at half the maximum signal intensity

Note 1 to entry: It is expressed in Hz.

[SOURCE: ISO 24583:2022, 3.9 – modified: Note 2 to entry deleted]

3.6**kahweol**

1,2-Dehydrocafestol (CAS RN®: 6894-43-5)

Note 1 to entry: The substance appears in its free form or esterified, see Figure 1.

3.7**dehydrokahweol**

1,2,15,16-bis-dehydrocafestol (CAS RN®: not yet available)

Note 1 to entry: It is a roasting product from kahweol.

Note 2 to entry: The substance appears in its free form or esterified, see Figure 1.

3.8**NMR****nuclear magnetic resonance spectroscopy**

method based on the selective absorption of high frequency radio waves by atomic nuclei subjected to a stationary magnetic field

Note 1 to entry: NMR provides chemical and structural properties of molecules.

[SOURCE: ISO 23118:2021, 3.10]

3.9**ppm****part per million**

<NMR> unit for the chemical shift (δ) of a resonance signal

Note 1 to entry: Dividing a signal's real resonance frequency f_{res} (in Hz, cycles per second) by the frequency rating of the NMR spectrometer f_{spect} and multiplying with 10^6 , a normalized shift measure is obtained that is independent of the spectrometer's field strength. See the following formula for explanation:

$$\delta_{\text{res}} = \frac{f_{\text{res}}}{f_{\text{spect}}} \times 1\,000\,000$$

3.10**PULCON****Pulse-Length-based CONcentration determination**

quantitative NMR method with external standard factoring in the duration of the 90° excitation pulse into the calculation of concentrations

prEN 17992:2023 (E)**3.11****QuantRef****Quantification Reference**

external standard to determine the spectrometer's response (signal integral per amount of NMR-active, measured nuclei)

Note 1 to entry: The QuantRef shall be a stable solution of one or more certified reference substances showing at least one intensive and baseline-separated resonance in the NMR-spectrum. Preferably substances with short T_1 transversal relaxation value should be used, thus enabling the use of shorter recycle delays between FIDs. The concentration(s) should be high enough to yield an SNR > 150 (reducing the uncertainty due to noise under 1 %) under applied NMR acquisition parameters (typical concentrations should be between 10 to 50 mmol/L). Reference substances intended for use in a QuantRef shall have a proven long-lasting stability in this mixture (ambient light, normal room temperature) Signals of the reference substances should not be close to the residual non-deuterated solvent signal or a trace water signal. Care shall be taken to ensure signals are separated by a clear, flat baseline without signal overlap, e.g. from ^{13}C satellites.

3.12**sample temperature**

calibrated temperature of the sample during the NMR experiment(s)

3.13**SNR**

signal to noise ratio

4 Reagents**4.1 General requirements**

Only certified and traceable substances of highest purity shall be used as reference substances. These reference substances shall be stored separately from the samples.

4.2 Deuterated Chloroform (CDCl_3), minimum degree of deuteration 99,8 % (at.), CAS RN® 865-49-6

Deuterated solvent for the extraction and the NMR measurement of lipophilic substances in coffee.

4.3 Tetramethylsilane (TMS), CAS RN®: 75-76-3

Internal standard used for the calibration of the ppm-scale $\delta(\text{TMS}) = 0,00$ ppm (def). May be omitted if the solvent's residual non-deuterated signal is used for calibration of the chemical shift scale

4.4 Deuterated Chloroform with TMS

TMS is added to CDCl_3 to achieve a TMS content between 0,03 % and 0,1 %. The solution shall be stored in the dark, preferably in a brown glass bottle. Some centimetres of silver tape should be added to hinder the formation of aggressive compounds (phosgene, hydrogen chloride) that could later deteriorate the samples. The possible addition of molecular sieves (3 \AA or 4 \AA)³ helps to absorb any residual water.

4.5 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB), CAS RN®: 117-18-0, M = 260,89 g/mol

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 7,74 ppm, s, 1 H; very stable in CDCl_3 , but long T_1 transversal relaxation value.

4.6 Ethylbenzene, CAS RN®: 100-41-4, M = 106,17 g/mol

³ $1 \text{ \AA} = 10^{-10} \text{ m}$, molecular sieves of different pore sizes are generally offered in Ångström units.

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 7,30 ppm to 7,26 ppm, m, 2 H; 7,23 ppm to 7,15 ppm, m, 2 H; 7,23 ppm to 7,15 ppm, m, 1 H; 2,65 ppm, q (7,6 Hz), 2 H and 1,24 ppm, t (7,6 Hz), 3 H.

4.7 1,3,5-Trimethoxybenzene, CAS RN®: 621-23-8, M = 168,19 g/mol

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 6,1 ppm, s, 3 H and 3,8 ppm, s, 9 H.

4.8 p-Xylene, CAS RN®: 106-42-3, M = 106,2 g/mol

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 7,06 ppm, s, 4 H and 2,31 ppm, s, 6 H.

4.9 Dimethylsulfon, CAS RN®: 67-71-0, M = 94,13 g/mol

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 2,31 ppm, s, 6 H.

4.10 Cyclohexane, CAS RN®: 110-82-7, M = 84,16 g/mol⁴

Exemplary reference substance for the QuantRef solution, δ (in CDCl_3) = 1,43 ppm, s, 12 H. Caution: resonance close to water signal in CDCl_3 .

4.11 QuantRef solution

In a 5 mL volumetric flask at least one of the reference substances (4.5 to 4.10) is/ are dissolved in CDCl_3 to achieve concentrations between 5 to 10 mg/mL. The exact weighed portions shall be documented (6.3.1). After thorough mixing, fill 0,6 mL of this QuantRef solution into an NMR tube that subsequently is fused shut. By repeated weighing the tube shall be checked against leakage, the proven tube shall be labelled with a distinct ID and the date of preparation.

In general, other reference substances and concentrations may be used for the QuantRef solution as long as the conditions stated in 3.9 are fulfilled. By using more than one substance, a higher reliability of the QR solution can be reached.

4.12 Quality control sample for monitoring repeatability and reproducibility

To supervise the whole process regularly, an appropriately sized sample of real coffee with a substantial 16-OMD content shall be stored in a tightly closed vessel in the dark. From this quality control sample one aliquot shall be prepared and measured with each new sample series, the results shall be documented in a control chart. The mean (expected value) and the upper/ lower thresholds shall be based on an appropriate number of replicates (prior period). Warning thresholds of $\pm 2 \sigma$ and action thresholds of $\pm 3 \sigma$ are recommended. The current result should stay within warning thresholds and shall stay within action thresholds else the measurement batch should be repeated.

5 Apparatus

5.1 General

⁴ Chloroform can slowly deteriorate the coffee extract and is a hazardous solvent. Cyclohexane has been proven (elsewhere) to extract the 16-OMD as well as chloroform, thus it can be used as a substitute: Prepare a 95/5 mixture of non-deuterated/deuterated cyclohexane, use this in the extraction step, then centrifuge the suspension (centrifugation works well with cyclohexane, but not with chloroform). From the clear supernatant take a filtered 600 μL aliquot for NMR measurement. Adapt the NMR experiment to suppress the cyclohexane resonance (1,41 ppm, s), then proceed as usual. Cyclohexane's extraction capacity for other substances (HMF, furfuryl alcohol) can be unsatisfying. This modification would need individual validation.