SLOVENSKI STANDARD
oSIST prEN 16466-1:2023

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Pristnost živil - Izotopska analiza ocetne kisline in vode v kisu - 1. del: 2H-NMR-analiza ocetne kisline

Food authenticity - Isotopic analysis of acetic acid and water in vinegar - Part 1: 2H-NMR analysis of acetic acid

Lebensmittelauthentizität - Isotopenanalyse von Essigsäure und Wasser in Essig - Teil 1: 2H-NMR-Analyse von Essigsäure

Authenticité des aliments - Analyse isotopique de l'acide acétique et de l'eau dans le vinaigre - Partie 1 : Analyse RMN-2H de l'acide acétique

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English Version

Food authenticity - Isotopic analysis of acetic acid and water in vinegar - Part 1: 2H-NMR analysis of acetic acid

Authenticité des aliments - Analyse isotopique de l'acide acétique et de l'eau dans le vinaigre - Partie 1 : Analyse RMN-2H de l'acide acétique

Lebensmittelauthentizität - Isotopenanalyse von Essigsäure und Wasser - Teil 1: 2H-NMR-Analyse von Essigsäure

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.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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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 16466-1:2023) 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.

This document will supersede EN 16466-1:2013.

The main changes compared to the previous edition are listed below:

a) extension to matrices other than wine vinegar;

b) modification of the NMR sequence;

c) standard editorially revised.

Introduction

Vinegar is defined in EN 13188 [3] as the acetic acid solution resulting from a double fermentation:

a) transformation of sugars to ethanol; and

b) transformation of ethanol to acetic acid.

Conversely, EN 13189 [4] defines acetic acid as “Product made from materials of non-agricultural origin”.

Wine vinegar is defined by the European Regulations 1308/2013 [5] as the product obtained exclusively from the acetous fermentation of wine, which is in turn defined as the product exclusively obtained from the alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must. Similarly, cider vinegar is defined as the product obtained from the acetous fermentation of apple. Thus, the acetic acid part of these vinegars cannot be obtained from the fermentation of exogenous sugars (e.g. from beet or cane).

Regulation EC no 813/2000 [6] defines Aceto Balsamico Tradizionale di Modena and Aceto Balsamico Tradizionale di Reggio Emilia, as PDOs and Regulation EC no. 583/2009 [7] defines Aceto Balsamico di Modena as PGI.

In all types of vinegar, both the ethanol and the acetic acid should be obtained by a biotechnological process, and the use of acetic acids obtained from either petroleum derivatives or the pyrolysis of wood is not permitted according to the above definitions.

The isotopic analysis of acetic acid extracted from vinegar by ^{2}H-SNIF-NMR and ^{13}C-IRMS enables the distinction of grape origin from other sources, such as beet, cane, malt, apple and synthesis [8]. Isotopic methods have been recognized by the European Committee for Standardization (CEN) and in part by the Organization Internationale de la Vigne et du Vin (OIV) as a means of detecting the non-permitted presence of exogenous acetic acid and water in vinegar (CEN) and specifically wine vinegar (OIV). The methods used are EN 16466-1 for D/H in the methyl site of acetic acid [$(D/H)_{CH3}$] using ^{2}H-SNIF-NMR (Site Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance), EN 16466-2 and OIV 510/2013 for analysis of $^{13}C/^{12}C$ in acetic acid using IRMS (Isotope Ratio Mass Spectrometry), and EN 16466-3 and OIV 511/2013 for analysis of $^{18}O/^{16}O$ in water using IRMS. Recently, it was experimentally proven that OIV and CEN methods are also applicable to the analysis of acetic acid extracted from Aceto Balsamico di Modena (D/H, ^{13}C) [9].

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 should 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.

This document has been based on an international collaborative study of the methods published in Molecules 2020, 25, 2932 [1]; and is an update and extension of the previous version published in 2013 after a first an international collaborative study published in Analytica Chimica Acta 649 (2009) 98-105 [2], and organized under the auspices of the Permanent International Vinegar Committee (CPIV, Brussels).

1 Scope

This document specifies an isotopic method to control the authenticity of vinegar and food containing vinegar as an ingredient (for example Aceto Balsamico di Modena), with a density below 1,28 g/cm^3. This method is applicable on acetic acid of vinegar (from wine, cider, agricultural alcohol, etc.) in order to characterize the botanical origin of acetic acid and to detect adulterations of vinegar using synthetic acetic acid or acetic acid from a non-allowed origin (together with the method described in EN 16466-2).

The isotopic analysis of the extracted acetic acid by ^{2}H-NMR is based on a similar method already normalized for wine analysis [10].

The application of this document can involve the use of hazardous substances, operations and equipment. This document does not claim to address all associated safety issues. It is the responsibility of the user of this document to take appropriate measures for the safety and health protection of personnel before use, and to check the applicability of existing national and European rules and regulations.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

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/

4 Principle

The acetic acid from vinegar is first extracted with diethyl ether (or alternatively another solvent with similar properties such as tert-butyl methyl ether), using a liquid-liquid extractor, during at least 5 h. The solvent is then eliminated by distillation. The acetic acid extracted from the sample is then analysed by ^{1}H-NMR and ^{2}H-SNIF-NMR using a composite NMR experiment [10], [11]: the weight ratio between tetramethylurea (TMU) and acetic acid is determined by ^{1}H-NMR, and the isotopic ratio of hydrogen atoms at the methyl site of acetic acid, $(D/H)_{CH3}$, is determined by ^{2}H-SNIF-NMR (Nuclear Magnetic Resonance analysis of the Deuterium), on the same NMR tube.

5 Reagents

All reagents and consumables used shall meet stated requirements of the used method/apparatus (as specified by the manufacturer). However, all reagents and consumables can be replaced by items with similar performance.

5.1 Diethyl ether, for analysis. Purity ≥ 99 %.

5.2 Standard N,N-tetramethylurea (TMU), standard TMU with a calibrated isotope ratio D/H.

5.3 Hexafluorobenzene (C6F6), used as field-frequency stabilization substance (lock). Alternatively, trifluoroacetic acid (TFA, CAS: 76-05-1) or trifluoroacetic anhydride (TFAA, CAS: 407-25-0), or a mix of them (e.g. 10 mL C_6F_6 + 1 mL TFA).

6 Apparatus

All materials listed below are commercially available and used in food control laboratories:

6.1 For the extraction of acetic acid from vinegar

6.1.1 Liquid-liquid extractor of 400 ml or 800 ml.

6.1.2 Cadiot type (with spinning band) or Vigreux distillation column.

6.1.3 Round bottom flask of 500 ml.

6.1.4 Erlenmeyer of 250 ml.

6.1.5 Condenser.

6.1.6 Heating mantle.

6.1.7 Pumices stones.

6.1.8 Micropipettes enabling to take from 0,1 to 5 ml.

6.1.9 Fume hood.

6.2 For composite SNIF-NMR analysis of acetic acid from vinegar

6.2.1 Filter 0,45 µm.

6.2.2 NMR spectrometer fitted with a specific deuterium probe tuned to a frequency v0, characteristic of the field B0 (e.g. B0 = 7,05 T, v0 = 46,05 MHz and for B0 = 9,4 T, v0 = 61,4 MHz) with a decoupling channel (B2) and a field-frequency stabilization channel (lock) at the fluorine frequency. The second channel, B2, is also used for the 1H-NMR experiment.

The resolution measured on the spectrum for 2H experiment, transformed without exponential multiplication (i.e. LB = 0) and expressed as the width at the half-height of the methyl signals of acetic acid and the methyl signal of TMU shall be less than 0,7 Hz.

The sensitivity (signal-to-noise ratio), measured with an exponential multiplying factor LB equal to 2 Hz shall be greater than or equal to 150 for the methyl signal of acetic acid containing less than 25 % of water.

For example, using a NMR spectrometer of field B0 = 7,05 T, 200 scans are necessary to reach this value (depending on the noise of the instrument).

6.2.3 Automatic sample changer (optional).

6.2.4 Data-processing software enabling lorentzian integration.

6.2.5 10 mm sample tubes of sufficient quality for NMR spectrometer 400 MHz.

6.2.6 Fume hood.

7 Procedure

7.1 Extraction of acetic acid from vinegar

7.1.1 General

This extraction procedure is only applicable to sample with a density below 1,25g/cm^3. For samples with higher density, the sample shall be diluted and the volume shall be adapted consequently.

7.1.2 Liquid-liquid extraction

A liquid-liquid extractor is illustrated in Figure 1.

Figure 1 — Liquid-liquid extractor

Put 125 ml of diethyl ether into a 250 ml round bottom flask. Use a 400 ml or a 800 ml liquid-liquid extractor, depending on the acetic acid content of the vinegar. At least 6 ml of pure acetic acid shall be recovered at the end of the extraction.

Pour approximately 400 ml of the sample into the extractor and top up with diethyl ether. Adapt the round bottom flask of the extractor and place it in the heating mantle. Open the water for the condenser, which is located in the upper part of the extractor, and switch the heater on. The extraction shall last at least 5 h after reflux start.

After this time period, separate the aqueous and the organic solution contained into the extractor. Recover the organic solution from the extractor and add it to the extract in the round bottom flask, previously tared.

NOTE If small part of aqueous phase has been recovered together with the organic solution, it is possible to dry with anhydrous sodium sulfate, and then filter through cotton or paper filter.

7.1.3 Purification of the extract

The round bottom flask containing the acetic acid in solution in diethyl ether is distilled on Cadiot (spinning band, as illustrated below in Figure 2) or Vigreux column.