BS EN 16466-2:2013

BSI Standards Publication

Vinegar — Isotopic analysis of acetic acid and water

Part 2: ^{13}C-IRMS analysis of acetic acid

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Foreword

This document (EN 16466-2:2013) has been based on an international collaborative study of the methods published in Analytica Chimica Acta 649 (2009) 98-105, and organised under the auspices of the Permanent International Vinegar Committee (CPIV, Brussels).

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of an identical text or by endorsement, at the latest by July 2013, and conflicting national standards shall be withdrawn at the latest by July 2013.

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

The European standard, *Vinegar — Isotopic analysis of acetic acid and water*, consists of the following parts:

— *Part 1: ^{2}H-NMR analysis of acetic acid*;

— *Part 2: ^{13}C-IRMS analysis of acetic acid*;

— *Part 3: ^{18}O-IRMS analysis of water*.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Vinegar is defined by EN 13188 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 defines acetic acid as "Product made from materials of non-agricultural origin".

Wine vinegar is defined by the European Regulations 479/2008 and 491/2009 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.

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 [1].

1 Scope

This European Standard specifies an isotopic method to control the authenticity of vinegar. This method is applicable on acetic acid of vinegar (from cider, alcohol, wine, etc.) in order to characterise the botanical origin of acetic acid and to detect adulterations of vinegar using synthetic acetic acid or acetic acid from not allowed origin (together with the method described in EN 16466-1).

The isotopic analysis of the extracted acetic acid by ^{13}C-IRMS is based on a similar method already normalised for wine analysis [2].

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Not applicable.

3 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 $^{13}C/^{12}C$ ratio of acetic acid from vinegar is then determined by Isotope Ratio Mass Spectrometry (IRMS) on the CO_2 gas resulting from a complete combustion at high temperature in an Elemental Analyser.

4 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.

4.1 Diethyl ether

For analysis.

4.2 Carbon dioxide

For analysis, used as secondary reference gas for the determination of $^{13}C/^{12}C$ ratio. Purity 5.2 minimum.

4.3 Helium

For analysis. Purity 5.6 minimum.

4.4 Oxygen

For analysis. Purity 5.0 minimum.

4.5 Oxidation reagent

For the furnace of the combustion system, like copper oxide, cobalt oxide...

4.6 Desiccant

To eliminate water produced in combustion if necessary, such as magnesium perchlorate

5 Apparatus

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

5.1 For the extraction of acetic acid from vinegar

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

5.1.2 Spinning band or Vigreux column.

5.1.3 Round bottom flask of 500 ml.

5.1.4 Erlenmeyer of 250 ml.

5.1.5 Condenser.

5.1.5 Heater.

5.2 For the determination of the isotopic ratio $^{13}C/^{12}C$ of acetic acid from vinegar

5.2.1 Isotope Ratio Mass spectrometer with an internal repeatability of 0,05‰.

5.2.2 Triple collector for simultaneous recording of ions m/z 44, 45 and 46.

5.2.3 Dual Inlet or Conflo to introduce alternatively reference CO_2 gas and CO_2 produced by sample combustion.

5.2.4 Elemental Analyser to carry out the complete combustion of organic products into CO_2 gas and equipped with a water trap.

5.2.5 Tin or silver capsules for liquid samples or liquid injectors systems.

5.2.6 Tweezers for encapsulation.

5.2.7 Eppendorf pipette with plastic disposable tip.

6 Procedure

6.1 Extraction of acetic acid from vinegar

6.1.1 Liquid-liquid extraction

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 the vinegar into the extractor and complete with diethyl ether. Adapt the round bottom flask, open the water for the condenser and switch the heater on. The extraction shall last at least 5 h.

Then, after this time, separate the aqueous and the organic solution. Recover the organic solution from the extractor and add it to the extract in the round bottom flask.

6.1.2 Purification of the extract

The round bottom flask containing the acetic acid in solution in diethyl ether is distilled on spinning band or Vigreux column.

An appropriate 250 ml Erlenmeyer is used to collect the distillate.

Open the water for the condenser and switch the heater on. The heating shall be weak during the distillation of the solvent (boiling point of diethyl ether 34 °C). When the main part of the solvent has been distilled (no more vapours at the head of the column), increase the heating.

The distillation is completed when the temperature is stable at about 98 °C (pure acetic acid distils at 116 °C to 117 °C).

6.2 Determination of the isotopic ratio $^{13}C/^{12}C$ of acetic acid from vinegar

6.2.1 Experimental determinations

Place the samples in capsules (the appropriate quantity of acetic acid shall be calculated according to the quantity of carbon necessary given the sensitivity and the linearity of the mass spectrometry apparatus used). Each capsule shall be completely sealed. At least 2 capsules shall be prepared for every sample. Place the capsules in the appropriate place on the tray of the automatic sampler of the elemental analyser. Place systematically capsules containing working references at the beginning and at the end of the sample series, and insert regularly control samples.

Check the IRMS instrument and adjust it for optimal combustion: furnace temperature, helium and oxygen flows. Check the system for leaks. Adjust the IRMS to measure the ionic currents m/z = 44, 45 and 46. Check the accuracy of the system using known control samples before starting to measure the samples.

The samples placed on the auto sampler of the elemental analyser are introduced in turn. The CO_2 from each sample combustion is eluted towards the mass spectrometer which measures the ionic currents. The software records the 3 ionic currents and calculates the isotopic ratio $^{13}C/^{12}C$ for each sample.

6.2.2 Calculation and expression of the results

The purpose of the method is to measure the $^{13}C/^{12}C$ ratio of acetic acid extracted from vinegar. The $^{13}C/^{12}C$ isotope ratio can be expressed by its deviation from a working reference. The isotopic deviation of carbon 13 ($\delta^{13}C$) is then calculated on a delta scale per thousand (‰) by comparing the results obtained for the sample to be measured with those for a working reference previously calibrated on the basis of the primary international reference (V-PDB). The $\delta^{13}C$ values (in ‰) are expressed in relation to the working reference as follows:

$$\delta^{13}C = \frac{R\left(^{13}C/^{12}C\right)_{sample} - R\left(^{13}C/^{12}C\right)_{standard\,(V\text{-}PDB)}}{R\left(^{13}C/^{12}C\right)_{standard\,(V\text{-}PDB)}}$$

where R_{sample} and $R_{standard}$ are respectively the $^{13}C/^{12}C$ isotope ratios of the sample and of the standard.

Between two measurements of the standard working sample, the variation, and therefore the correction to be applied to the results obtained from the samples, may be assumed to be linear. The standard working sample

shall be measured at the beginning and at the end of all sample series. A correction can then be calculated for each sample according to its position in the sequence using linear interpolation.

7 Precision

7.1 Repeatability

In the collaborative study organised in 2009 (see Annex A: Results of the collaborative study (2009)), the average repeatability limit ($r = 2,8 \times s_r$) of the $\delta^{13}C$ of acetic acid was 0,45 ‰.

7.2 Reproducibility

In the collaborative study organised in 2009 (see Annex A: Results of the collaborative study (2009)), the average reproducibility limit ($R = 2,8 \times s_R$) of the $\delta^{13}C$ of acetic acid was 0,91 ‰.

8 Test report

The test report shall contain at least the following:

a) all information necessary for the identification of the sample;

b) a reference to this European Standard or to the method used;

c) the results and the units in which the results have been expressed;

d) date and type of sampling procedure (if known);

e) date of receipt;

f) date of test;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Results of the collaborative study (2009)

The samples have been prepared using the materials listed in Table A.1, plus a mixture of samples B and C. All samples have been analysed as blind duplicates, so in total twelve samples have been sent to each participating laboratories. Following the IUPAC internal harmonised protocol for collaborative studies [3], this experimental design intends to have more than five "materials", i.e. different matrix / sample pairs.

Homogeneity tests have been performed by the coordinator before shipment of samples. The statistical tests performed according to [4] confirmed a sufficient homogeneity for the six sample pairs.

Statistical calculations have then been performed according to ISO 5725-4 [5] and the IUPAC protocol [3]. Outliers have been removed in the following way: a loop of Cochran tests for removal of laboratories with highest variance, single and pair value Grubbs tests for individual or paired individual outliers, then back to Cochran test, etc., keeping a proportion of outliers <2/9. Then the standard deviations of repeatability (s_r) and of reproducibility (s_R) for each material have been computed from valid results pairs on the blind duplicates. A summary of these calculations are presented in Table A.2.

Table A.1 — Bulk materials used to prepare the samples

Material code	Description	Acetic acid content %
A	Vinegar from cider	4,9
B	Alcohol vinegar	7,8
C	Red wine vinegar n° 1	7,0
D	White wine vinegar	6,0
E	Red wine vinegar n°2	7,0

Table A.2 — Summary of statistics for 13C-IRMS results

Sample description	A	B	C	D	C+20%B	E
Number of valid results	11	10	11	10	11	7
Number of replicates	2	2	2	2	2	2
Mean $\delta^{13}C$ (‰)	-29,16	-29,52	-26,66	-27,51	-27,15	-27,19
s_r (‰)	0,21	0,14	0,11	0,20	0,14	0,33
s_R (‰)	0,41	0,34	0,25	0,33	0,29	0,46

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[6] EN 13188, *Vinegar — Product made from liquids of agricultural origin — Definitions, requirements, marking*

[7] EN 13189, *Acetic acid food grade — Product made from materials of non-agricultural origin — Definitions, requirements, marking*

[8] COUNCIL REGULATION (EC) No 479/2008 of 29 April 2008 on the common organisation of the market in wine, amending Regulations (EC) No 1493/1999, (EC) No 1782/2003, (EC) No 1290/2005, (EC) No 3/2008 and repealing Regulations (EEC) No 2392/86 and (EC) No 1493/1999, L 148/1 –61

[9] COUNCIL REGULATION (EC) No 491/2009 of 25 May 2009 amending Regulation (EC) No 1234/2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation), L154/1-56

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