BS EN 16466-3:2013

BSI Standards Publication

Vinegar — Isotopic analysis of acetic acid and water

Part 3: ^{18}O-IRMS analysis of water in wine vinegar

...making excellence a habit.™

National foreword

This British Standard is the UK implementation of EN 16466-3:2013.

The UK participation in its preparation was entrusted to Technical Committee AW/-/2, Food Technical Committee Chairmen.

A list of organizations represented on this committee can be obtained on request to its secretary.

Published by BSI Standards Limited 2013

ISBN 978 0 580 73668 1

ICS 67.220.10

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 January 2013.

Amendments issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16466-3

January 2013

ICS 67.220.10

English Version

Vinegar - Isotopic analysis of acetic acid and water - Part 3: ^{18}O-IRMS analysis of water in wine vinegar

Vinaigre - Analyse isotopique de l'acide acétique et de l'eau - Partie 3: Analyse SMRI-^{18}O de l'eau dans le vinaigre de vin

Essig - Isotopenanalyse von Essig und Wasser - Teil 3: ^{18}O-IRMS-Analyse von Wasser in Weinessig

This European Standard was approved by CEN on 3 November 2012.

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. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists 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.

CEN members are the national standards bodies of 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 United Kingdom.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 16466-3:2013: E

Contents

Page

Foreword

This document (EN 16466-3:2013) has been based on an international collaborative study of the method 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

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 accordance with this, it is clear that the production of wine vinegar by fermenting dried grapes and rehydrating with tap water is not allowed by European Regulations.

The isotopic analysis of water from vinegar by ^{18}O-IRMS enables the distinction of wine vinegar and vinegars from fermented dried grapes which have been rehydrated with water [1], and more generally helps to check the authenticity of wine vinegar.

1 Scope

This European Standard specifies an isotopic method to control the authenticity of wine vinegar. This method is applicable on wine vinegar in order to characterise the $^{18}O/^{16}O$ ratio of water, and allows differentiating wine vinegar from vinegars made from raisins or alcohol vinegar.

NOTE The Oxygen 18 isotopic analysis of water from vinegar 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 $^{18}O/^{16}O$ ratio of water from vinegar is determined on CO_2 gas after equilibration of reference CO_2 gas with vinegar water according to the following isotopic exchange reaction:

$$C^{16}O_2 + H_2{}^{18}O \leftrightarrow C^{16}O^{18}O + H_2{}^{16}O \quad (1)$$

After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the $^{18}O/^{16}O$ isotopic ratio is determined on the CO_2 resulting from the equilibration.

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 Carbon dioxide

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

4.2 Carbon dioxide used for equilibration

Depending on the instrument, this gas could be the same as 4.1 or in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used

5 Apparatus

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

5.1 Vials with septa appropriate for the used system.

5.2 Volumetric pipettes with appropriate tips.

5.3 Temperature controlled system to carry out the equilibration at constant temperature, typically within ± 1 °C.

5.4 Vacuum pump (if needed for the used system).

5.5 Autosampler (if needed for the used system).

5.6 Syringes for sampling (if needed for the used system).

5.7 GC Column to separate CO_2 from other elementary gases (if needed for the used system).

5.8 Water removal device (e.g. cryo-trap, selective permeable membranes).

6 Procedure

6.1 General

The descriptions that follow refer to procedures generally used for the determination of the $^{18}O/^{16}O$ isotopic ratios by means of equilibration of water with a CO_2 working standard and the subsequent measurement by IRMS. These procedures can be altered according to changes of equipment and instrumentation provided by the manufacturers as various kinds of equilibration devices are available, implying various conditions of operation. Two main technical procedures can be used for introduction of CO_2 into the IRMS either through a dual inlet system or using a continuous flow system. The description of all these technical systems and of the corresponding conditions of operation is not possible.

All values given for volumes, temperatures, pressures and time periods are only indicative. Appropriate values shall be obtained from specifications provided by the manufacturer and/or determined experimentally.

6.2 Manual method

A defined volume of the sample/standard is transferred into a flask using a pipette. The flask is then attached tightly to the manifold. Each manifold is cooled down to below – 80 °C to deep-freeze the samples (manifold equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated. After reaching a stable vacuum the gaseous CO_2 working standard is allowed to expand into the various flasks. For the equilibration process each manifold is placed in a temperature controlled waterbath typically at 25 °C (± 1 °C) for 12 h (overnight). It is crucial that the temperature of the water-bath is kept constant and homogeneous.

After the equilibration process is completed, the resulting CO_2 is transferred from the flasks to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.

This approach is repeated till the last sample of the sequence has been measured.

6.3 Use of an automatic exchange apparatus

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are attached to the equilibration system and cooled down to below – 80 °C to deep-freeze the samples (systems equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated.

After reaching a stable vacuum the gaseous CO_2 working standard is expanded into the vials. Equilibrium is reached at a temperature of typically (22 ± 1) °C after a minimum period of 5 h and with moderate agitation (if available). Since the equilibration duration depends on various parameters (e.g. the vial geometry, temperature, applied agitation ...), the minimum equilibrium time should be determined experimentally.

After the equilibration process is completed, the resulting CO_2 is transferred from the vials to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.

This approach is repeated till the last sample of the sequence has been measured.

6.4 Manual preparation manual and automatic equilibration and analysis with a dual inlet IRMS

A defined volume of sample / standard (e.g. 200 µl) is introduced into a vial using a pipette. The open vials are then placed in a closed chamber filled with the CO_2 used for equilibration (4.2). After several purges to eliminate any trace of air, the vials are closed and then placed on the thermostated plate of the sample changer. The equilibration is reached after at least 8 h at 40 °C. Once the process of equilibration completed, the CO_2 obtained is dried and then transferred into the sample side of the dual inlet introduction system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.

This approach is repeated until the last sample of the sequence has been measured.

6.5 Use of an automatic equilibration apparatus coupled to a continuous flow system

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are placed into a temperature controlled tray.

Using a gas syringe the vials are flushed with mixture of He and CO_2. The CO_2 remains in the headspace of the vials for equilibration. Equilibrium is reached at a temperature typically of (30 ± 1) °C after a minimum period of 18 h. After the equilibration process is completed the resulting CO_2 is transferred by means of the continuous flow system into the ion source of the mass spectrometer. CO_2 reference gas is also introduced into the IRMS by means of the continuous flow system. The measurement is carried out according to a specific protocol for each kind of equipment.

6.6 Calculation and expression of the results

The purpose of the method is to measure the $^{18}O/^{16}O$ ratio of water extracted from vinegar. The $^{18}O/^{16}O$ isotope ratio can be expressed by its deviation from a working reference. The isotopic deviation of oxygen 18 ($\delta^{18}O$) 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.SMOW2). The $\delta^{18}O$ values (in ‰) are expressed in relation to the working reference as follows:

$$\delta^{18}\mathrm{O} = \frac{R\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_{\mathrm{sample}} - R\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_{\mathrm{standard\,(V.SMOW2)}}}{R\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_{\mathrm{standard\,(V.SMOW2)}}} \quad (2)$$

where R_{sample} and $R_{standard}$ are respectively the $^{18}O/^{16}O$ 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 $\delta^{18}O$ of water was 0,15 ‰.

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 $\delta^{18}O$ of water was 0,59 ‰.

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 18O-IRMS results

Sample description	A	B	C	D	C+20%B	E
Number of valid results	8	8	8	8	8	5
Number of replicates	2	2	2	2	2	2
Mean $\delta^{18}O$ (‰)	-4,53	-6,96	-0,86	-1,38	-1,79	-1,23
s_r (‰)	0,06	0,06	0,08	0,02	0,05	0,05
s_R (‰)	0,22	0,22	0,21	0,20	0,20	0,14

Bibliography

[1] F. Camin., L. Bontempo, M. Perini, A. Tonon, O. Breas, C. Guillou, J.M. Moreno-Rojas, G. Gagliano. Control of wine vinegar authenticity through δ18O analysis. Food Control, 29 (2013) 107-111

[2] OIV-MA-AS2-12, Method for $^{18}O/^{16}O$ isotope ratio determination of water in wines and must (Resolution OIV-Oeno 353/2009)

[3] Protocol for the design, conduct and interpretation of method-performance studies, W. Horwitz, Pure and Applied Chemistry, 1995, Volume 67, No. 2, pp. 331-343

[4] The international harmonized protocol for the proficiency testing of analytical chemistry laboratories, Thompson M., Ellison S. L. R. and Wood R., Pure Appl. Chem., 2006, 78, 145

[5] ISO 5725-4:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 4: Basic methods for the determination of the trueness of a standard measurement method*

[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

This page deliberately left blank

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards -based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Copyright

All the data, software and documentation set out in all British Standards and other BSI publications are the property of and copyrighted by BSI, or some person or entity that owns copyright in the information used (such as the international standardization bodies) and has formally licensed such information to BSI for commercial publication and use. Except as permitted under the Copyright, Designs and Patents Act 1988 no extract may be reproduced, stored in a retrieval system or transmitted in any form or by any means – electronic, photocopying, recording or otherwise – without prior written permission from BSI. Details and advice can be obtained from the Copyright & Licensing Department.

Useful Contacts:

Customer Services
Tel: +44 845 086 9001
Email (orders): orders@bsigroup.com
Email (enquiries): cservices@bsigroup.com

Subscriptions
Tel: +44 845 086 9001
Email: subscriptions@bsigroup.com

Knowledge Centre
Tel: +44 20 8996 7004
Email: knowledgecentre@bsigroup.com

Copyright & Licensing
Tel: +44 20 8996 7070
Email: copyright@bsigroup.com

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

bsi.

...making excellence a habit.™