RESOLUTION OIV-OENO 691-2025

DETERMINATION OF TARTARIC ACID (L+) ISOTOPIC RATIOS $^{13}C/^{12}C$ AND $^{18}O/^{16}O$ BY ISOTOPE RATIO MASS SPECTROMETRY

THE GENERAL ASSEMBLY,

IN VIEW OF ARTICLE 2, paragraph 2 iv of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

DECIDES, at the proposal of Commission II "Oenology", to amend the monograph COEI-1-LTARAC of Chapter I of the *International Oenological Codex* by adding the following sentence in italic under part 6 Identifying Characteristics: *"6.5 Determination of the source: The method described in Appendix I can be used to determine the origin of tartaric acid from grapes or synthetic."*

DECIDES, at the proposal of Commission II "Oenology", to add the following method as Appendix I to the monograph COEI-1-LTARAC of Chapter I of the *International Oenological Codex*:

APPENDIX I

DETERMINATION OF TARTARIC ACID (L+) ISOTOPIC RATIOS $^{13}C/^{12}C$ AND $^{18}O/^{16}O$ BY ISOTOPE RATIO MASS SPECTROMETRY

INTRODUCTION

Tartaric acid (L+) is a natural crystalline compound present in many fruits, in particular grape musts, where it varies from 0.2 to 6 g/L.

In the literature, it is shown that the isotope ratios $^{13}C/^{12}C$ and $^{18}O/^{16}O$ of tartaric acid can differentiate the tartaric acid of grape origin from the synthetic one.

The method described here can be used to determine the origin of tartaric acid: from grapes or synthetic.

WARNING: Some reagents used in this procedure are dangerous: take particular care

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when using them. The operator is advised to observe the instructions on the product container label, to consult the safety data sheets for specific information on the danger of the reagents used and on how to dispose of them.

1. PURPOSE AND SCOPE OF APPLICATION

The aim of the method is to analyse the isotope ratios $^{13}C/^{12}C$ and $^{18}O/^{16}O$ of tartaric acid (L+) with a purity of no less than 95% through the Isotope Ratio Mass Spectrometry (IRMS) technique.

2. TERMS AND DEFINITIONS

For the purposes of this document, the following definitions apply:

$^{13}C/^{12}C$: Ratio of carbon-13 to carbon-12 isotopes for a given sample.

$\delta^{13}C$: Ratio between carbon-13 (^{13}C) and carbon-12 (^{12}C) isotopes expressed in delta permil (‰).

$^{18}O/^{16}O$: Ratio of oxygen-18 to oxygen-16 isotopes for a given sample.

$\delta^{18}O$: Ratio between oxygen-18 and oxygen-16 isotopes expressed in delta permil (‰).

V-PDB (Vienna-Pee Dee Belemnite): International reference standard for the calculation of $\delta^{13}C$.

V-SMOW (Vienna-Standard Mean Ocean Water): International reference standard for the calculation of $\delta^{18}O$.

3. PRINCIPLE

The raw tartaric acid (L+) with a minimum purity of 95% is analysed. The stable isotope ratios of C and O are analysed using an Isotope Ratio Mass Spectrometer (IRMS) interfaced with an elemental analyser (EA) and a pyrolizer (P) starting from the ionic currents:

- m/z = 44 ($^{12}C^{16}O^{16}O$), m/z = 45 ($^{13}C^{16}O^{16}O$) produced by the carbon dioxide obtained from the combustion of the sample in the elemental analyser
- m/z = 28 ($^{12}C^{16}O$) and m/z = 30 ($^{12}C^{18}O$) produced by the carbon monoxide obtained from the pyrolysis of the sample in the pyrolizer.

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4. REAGENTS AND MATERIALS

The reagents and consumables depend on the equipment used by the laboratory. Elemental analysers are generally used for the combustion of the sample, while pyrolizers are generally used for the pyrolysis of the sample. These systems can be equipped with automatic systems for the introduction of samples placed in sealed metal capsules.

The laboratory may use any certified international reference material in addition to those indicated in the table in 4.3.

4.1. Consumables

4.1.1. Helium for analysis (CAS 07440-59-7);

4.1.2. Oxygen for analysis (CAS 07782-44-7);

4.1.3. Oxidation, reduction and pyrolysis reagents for the furnace and the combustion system such as copper oxide (II) for elemental analysis (CAS 1317-38-0) or glassy carbon for pyrolysis (CAS No 16291-96-6);

4.1.4. Column of alumina Al_2O_3 and glassy carbon for pyrolysis;

4.1.5. Desiccant to eliminate the water produced by combustion. For example: anhydrone for elemental analysis (magnesium perchlorate) (CAS 10034-81-8). Not necessary in the case of equipment equipped with a water elimination system through a cryogenic or capillary trap that is selectively permeable.

4.1.6. Disposable tin and silver capsules.

4.2. Working standards

4.2.1. Carbon dioxide (CO_2) (CAS 00124-38-9) with a purity of no less than 99.998% and carbon monoxide (CO) (CAS 630-08-0) with a purity of no less than 99.970% as reference gas for measurement.

4.2.2. Working and control standards of tartaric acid L(+) with values □□of $\delta^{13}C$ and $\delta^{18}O$ calibrated with respect to international reference materials.

4.3. Most commonly used reference materials

Name	Material	$\delta^{13}C$ vs V-PDB ‰	$\delta^{18}O$ vs V-SMOW ‰

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CBS	Caribou Hoof Standard		+2.39 ± 0.13
IAEA-600	Caffeine	-27.771 ± 0.043	
IAEA-601	Benzoic Acid	-28.81 ± 0.04	+23.14 ± 0.19
IAEA-602	Benzoic Acid	-28.85 ± 0.04	+71.28 ± 0.36
IAEA-CH-7	Polyethylene	-32.151 ± 0.05	
KHS	Kudu Horn Standard		+21.21 ± 0.17
NBS22	Mineral oil	-30.031 ± 0.04	
USGS40	Glutamic acid	-26.39 ± 0.04	
USGS24	Graphite	-16.05 ± 0.07	
USGS42	Tibetan human hair powder	-21.09 ± 0.10	+8.56 ± 0.10
USGS43	Indian human hair powder	-21.28 ± 0.10	+14.11 ± 0.10
USGS54	Canadian lodgepole pine wood powder	-24.43 ± 0.02	+17.79 ± 0.15
USGS55	Mexican ziricote wood powder	-27.13 ± 0.02	+19.12 ± 0.07
USGS56	South African red ivorywood powder	-24.34 ± 0.01	+27.23 ± 0.03
USGS90	Millet flour from Tuscany, Italy	-13.75 ± 0.06	+35.90 ± 0.29

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5. EQUIPMENT

Normal laboratory equipment, and in particular the following equipment:

5.1. Isotope Ratio Mass Spectrometer (IRMS)

Isotope ratio mass spectrometer allows the determination of the relative contents of the heavy isotope compared to the light one of the CO_2 and CO gas obtained from the combustion or pyrolysis of the sample, with an internal precision of 0.1‰ for C, 0.2‰ for O Internal precision is defined here as the difference between two measurements of the same gas sample.

The mass spectrometer used is generally equipped with a series of collectors to measure the ionic currents m/z = 44, 45, 46 at the same time; or m/z = 28, 29, 30.

By measuring the corresponding intensities, for example the $^{13}C/^{12}C$ isotope ratio is determined by the intensity ratio of m/z = 45 and m/z = 44 after corrections for the isobaric species $^{12}C^{17}O^{16}O$, whose contribution can be calculated as a function of the intensity of the current measured for m/z = 46 and of the relative abundance of ^{18}O and ^{17}O (Craig's correction).

The mass spectrometer for the determination of isotope ratios must be equipped with:

- A double introduction system (double inlet system) to alternately measure the sample and a reference standard.
- Or a continuous flow system that quantitatively transfers the gases resulting from the combustion of samples and working standards into the mass spectrometer.

5.2. Elemental Analyser (EA) or Pyrolizer (P)

Combustion and pyrolysis equipment capable of quantitatively converting the sample into carbon dioxide (in case of combustion) and carbon monoxide (in case of pyrolysis) and capable of separating gases and eliminating water without any isotope fractionation. The equipment can be either a continuous flow system integrated into the mass spectrometer, or an autonomous combustion system. In the latter case, the gases are collected in special containers which are then interfaced to the IRMS.

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5.3. Microanalytical balance

(min. range 0-100 mg and min. precision 0.01 mg) to measure the mass of the sample being analysed (see 6.2.1).

6. PROCEDURE

6.1. Preparation of the sample for analysis

The analysis is carried out on the tartaric acid sample as it is with a minimum purity of 95%. The samples are ground in a mortar to make them homogeneous.

6.2. Analysis of isotope ratios

For instrumental measurement, the procedure indicated in the instrument manual and recommended by the manufacturer's technicians is followed.

The following description refers to the procedure generally used for the combustion or pyrolysis of samples by means of commercial automated combustion systems. It is possible to use other methods, which ensure the quantitative conversion of the samples into carbon dioxide and carbon monoxide without loss by evaporation.

6.2.1. Inserting the samples into the capsules and analysis:

- Use clean capsules, tweezers and a prep shelf.
- Use Sn capsules for combustion and Ag for pyrolysis.
- Take a capsule of suitable size with tweezers.
- Introduce the required amount of sample into the capsule.

Note:

It is necessary to set the correct amount of sample to be weighted, such that the amount of CO (when considering the pyrolysis) or CO_2 (when considering the combustion) produced by the sample and the working standard (or reference material) do not differ for more than 50%. To fall within this range of acceptability, it is necessary to perform a preliminary measurement revealing the amount of sample to be weighed (if unknown).

- Weigh using microanalytical scale.

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- Close the capsule with tweezers.
- Prepare at least two capsules for each sample if analysing the 13C/12C isotope ratio and three if analysing 18O/16O.
- Properly arrange the capsules in the sample drum of the elemental analyser and/or pyrolizer (if available). Each capsule must be carefully identified in numerical order.
- Systematically arrange the capsules containing the working standard at the beginning and at the end of the series of samples.
- Regularly insert control samples into the sample set (e.g., a commercial tartaric acid previously calibrated, see 4.2) to build a control chart.

6.2.2. Control and adjustment of the instrumentation for isotope analysis

- For optimal combustion/pyrolysis of the sample, adjust the temperature of the furnaces of the elemental analyser/pyrolizer and the gaseous carbon dioxide, helium and oxygen flows following the manufacturer's instructions.
- Verify the absence of leaks in the system for elemental analysis/pyrolysis and mass spectrometry (for example, by checking the ionic current for the ratio m/z = 40 which corresponds to argon).
- Adjust the mass spectrometer following the manufacturer's instructions.
- Before starting measurements on samples, check the system using working standard samples.

6.2.3. Performing the measurements

- The samples are introduced in succession into the autosampler of the elemental analyser/pyrolizer. The gases produced by the combustion of each sample are transported in succession to the mass spectrometer which measures the ionic currents. The computer, interfaced with the instrumentation, records the intensity of the ionic currents and calculates the δ values for each sample.

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7. CALCULATION

7.1. Correction and expression of the isotope data

According to the IUPAC protocol, the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ values are expressed on the delta scale (□‰) with respect to the international standard V-PDB (Vienna-Pee Dee Belemnite) and V-SMOW (ocean water) according to equation 1:

$$(1)\delta_{ref}({}^{i}E/{}^{j}E,sample) = \left[\frac{R({}^{i}E/{}^{j}E,sample)}{R({}^{i}E/{}^{j}E,ref)}\right] - 1$$

where *ref* is the international measuring standard; *sample* is the sample analysed; ${}^{i}E/{}^{j}E$ is the isotope ratio between heavier and lighter isotopes. The delta values are then multiplied by 1000 and expressed in units permil (‰).

The data are usually provided by the instrument and refer to the standard gases used for analysis (CO_2 and CO). The data obtained must be corrected and normalised. At least two international reference materials (see table 4.3) or working standards (previously calibrated) must be placed at the beginning and end of the analytical sequence and have certified values at the extremes of the measurement range, both for the carbon analysis (e.g., USGS 24 and 55) and for the oxygen analysis (e.g., IAEA 601 and 602). The two points provided by the reference materials (or working standards) are used to create an interpolation line and calculate the relevant equation, which is then used to correct all the data obtained.

7.2. Quality control of the analyses

- The mean value obtained for the working or international standards used by the laboratory must fall within the validity range established by the laboratory during calibration or reported in the certificate.
- The difference between the two repeated measurements of the same sample must be less than 0.3‰ for the carbon analysis and 0.5‰ for the oxygen analysis.

7.3. Estimation of the repeatability and reproducibility of the method

The interlaboratory study took place from June to September 2022 and, through the

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participation of nine different international laboratories, made it possible to estimate the repeatability and reproducibility of the method. The repeatability and reproducibility studies were conducted on five double-blind samples of L-tartaric acid (total of 10 samples) with different $\delta^{13}C$ e $\delta^{18}O$ values. Each laboratory was also provided with two international standards with certified values, CBS Caribou Hoof Standard (with a certified value of $\delta^{18}O$ = +2.39‰ and $\delta^{13}C$ = -22.63‰) and USGS90 millet flour (with a certified value of $\delta^{18}O$ = +35.90 ‰ and $\delta^{13}C$ = -13.75‰) for a two-point line data correction.
The study data were provided by:

1. Fondazione Edmund Mach – San Michele all'Adige (Trento) – Italy
2. Agenzia Dogane Monopoli - Laboratorio chimico di Torino – Torino - Italy
3. Imprint Analytics GmbH – Neutal - Austria
4. Hydroisotop GmbH - Stable isotope laboratory – Schweitenkirchen – Germany
5. Agroisolab GmbH – Jülich – Germany
6. Eurofins Authenticity Competence Centre – Nantes – France
7. TLR international laboratories - Ridderkerk – Nederland (only for carbon)
8. State scientific research Institute of the brewing and wine industry – Moscow – Russia
9. SGS Taiwan Ltd. – New Taipei City – Taiwan

Based on the results obtained (reported in Table 1 and 2 of Appendix 1), it is possible to estimate the following validation parameters.

$\delta^{13}C$/ ‰ vs V-PDB

Sample	tartaric 1	tartaric 2	tartaric 3	tartaric 4	tartaric 5
Number of valid results	9	9	9	9	9

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Number of repetitions	2	2	2	2	2
Mean	-26.49	-30.21	-23.14	-24.81	-22.71
Sr	0.09	0.03	0.03	0.04	0.02
SR	0.12	0.12	0.09	0.09	0.09

$\delta^{18}O$ / ‰ vs V-SMOW					
Sample	tartaric 1	tartaric 2	tartaric 3	tartaric 4	tartaric 5
Number of valid results	8	8	8	8	8
Number of repetitions	2	2	2	2	2
Mean	13.38	16.24	28.41	38.3	47.14
Sr	0.22	0.15	0.2	0.13	0.26
SR	0.83	0.69	0.74	0.84	1.08

7.4. Repeatability and reproducibility

Repeatability

The absolute difference between two individual results detected on an identical sample by an operator using the same equipment in the shortest possible time interval will exceed the repeatability limit r in no more than 5% of cases.

The accepted values of the relative standard deviation of repeatability (RSDr) are equal to 0.09‰ for the $\delta^{13}C$ parameter and 0.26‰ for the $\delta^{18}O$ parameter.

Reproducibility

The absolute difference between two individual results found on an identical sample reported by two laboratories will exceed the reproducibility R in no more than 5% of

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cases.

The accepted values of the relative standard deviation of reproducibility (RSDR) are equal to 0.12‰ for the $\delta^{13}C$ parameter and 1.08‰ for the $\delta^{18}O$ parameter.

8. ISOTOPIC CHARACTERISATION OF TARTARIC ACID (L+) OF GRAPE ORIGIN

From January 2019 to December 2023, a research activity was conducted at the Mach Foundation in San Michele all'Adige (TN) Italy, leading to the definition of the $\delta^{13}C$ and $\delta^{18}O$ limit values of 95% for an L(+)tartaric acid of grape origin. 81 samples of calcium tartrate were collected from different countries around the world (Italy, France, Spain, Australia and South America) which represented the main suppliers of the raw material to processing companies. The suppliers guaranteed that the samples derived from lees and pomace from wineries that did not use (where permitted) tartaric acid of synthetic origin. The samples were transformed into L(+)tartaric acid in the laboratory with a pilot plant that allows working with small quantities but technically identical to the industrial one. Samples were purified and analysed for $\delta^{13}C$ and $\delta^{18}O$ ratios.

8.1. $\delta^{13}C$ and $\delta^{18}O$ limiting values for tartaric acid (L+) of grape origin

The limited values reported are based on the experimental data in Perini et al. 2025.

The minimum threshold limit value of $\delta^{13}C$ (minimum value of the dataset) for an L(+)tartaric acid of grape origin is equal to -24.6‰.

The minimum threshold limit value of $\delta^{18}O$ (minimum value of the dataset) for an L(+)tartaric acid of grape origin is equal to +27.8‰.

8.2. Guide to the interpretation of isotopic data

Samples with an isotopic value $\delta^{13}C$ and $\delta^{18}O$ below the limiting value for tartaric acid (L+) of grape origin as defined in point 8.1 minus the uncertainty calculated as 2* RSDR (see section 7.4) are to be regarded as non-grape origin.

9. BIBLIOGRAPHY

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APPENDIX I.1

Table 1 shows the $\delta^{18}O$ results provided by the different laboratories with an indication of the Z-score.

Lab	Sample 1	Sample 7	Z-score	Sample 5	Sample 9	Z-score	Sample 2	Sample 6	Z-score
1	14.65	14.72	1.58	17.31	17.26	1.51	29.32	29.33	1.24
2	14.08	14.26	0.96	16.93	17.01	1.06	29.15	29.30	1.11
3	13.95	13.81	0.61	16.78	16.58	0.64	28.91	29.21	0.88

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4	13.03	13.00	-0.44	16.23	15.89	-0.26	28.06	27.51	-0.84
5	13.39	13.45	0.05	15.97	15.93	-0.41	28.03	28.04	-0.50
6	12.91	13.02	-0.50	15.76	15.70	-0.73	28.53	28.54	0.17
7	12.20	12.56	-1.20	15.08	15.38	-1.45	27.56	28.01	-0.84
8	12.13	12.89	-1.05	16.14	15.84	-0.36	27.61	27.39	-1,22
Mean		13.38			16.24			28.41	

Lab	Sample 3	Sample 10	Z-score	Sample 4	Sample 8	Z-score
1	39.29	39.10	1.07	47.77	47.79	0.59
2	39.08	39.12	0.96	47.99	48.05	0.81
3	38.90	39.20	0.90	48.27	48.32	1.07
4	37.36	37.39	-1.10	45.88	45.25	-1.46
5	37.31	37.35	-1.16	45.75	45.85	-1.24
6	38.73	38.89	0.61	48.01	47.86	0.73
7	37.91	38.21	-0.28	47.20	47.41	0.15
8	37.41	37.50	-1.01	46.84	46.06	-0.64
Mean		38.30			47.14	

Table 2 shows the $\delta^{13}C$ results provided by the different laboratories with an indication of the Z-score.

Lab	Sample 1	Sample 7	Z-score	Sample 5	Sample 9	Z-score	Sample 2	Sample 6	Z-score

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1	-26.43	-26.37	0.76	-30.11	-30.12	0.77	-23.07	-23.06	0.82
2	-26.45	-26.43	0.42	-30.11	-30.17	0.57	-23.12	-23.18	-0.14
3	-26.39	-26.36	0.97	-30.13	-30.08	0.85	-23.03	-23.07	0.99
4	-26.34	-26.72	-0.36	-30.09	-30.07	1.06	-23.06	-23.12	0.54
5	-26.53	-26.48	-0.14	-30.29	-30.36	-0.93	-23.11	-23.11	0.31
6	-26.70	-26.71	-1.86	-30.47	-30.45	-2.02	-23.35	-23.34	-2.34
7	-26.48	-26.50	-0.01	-30.23	-30.21	-0.08	-23.17	-23.17	-0.36
8	-26.51	-26.48	-0.06	-30.26	-30.23	-0.28	-23.13	-23.14	0.03
9	-26.42	-26.49	0.29	-30.19	-30.22	0.04	-23.11	-23.14	0.14
Mean		-26.50			-30.22			-23.15	

Lab	Sample 3	Sample 10	Z-score	Sample 4	Sample 8	Z-score
1	-24.77	-24.77	0.51	-22.61	-22.61	1.06
2	-24.91	-24.80	-0.48	-22.76	-22.74	-0.44
3	-24.74	-24.72	0.97	-22.64	-22.61	0.90
4	-24.77	-24.72	0.80	-22.71	-22.72	-0.07
5	-24.80	-24.81	0.10	-22.70	-22.69	0.15
6	-25.02	-24.99	-2.22	-22.95	-22.90	-2.31
7	-24.77	-24.78	0.45	-22.68	-22.66	0.41
8	-24.80	-24.81	0.10	-22.67	-22.69	0.31
9	-24.79	-24.88	-0.24	-22.67	-22.75	-0.01
Mean		-24.82			-22.72	

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