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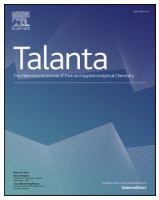

L(+)-tartaric acid of grape origin: Definition of threshold limits for the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ stable isotope ratios and validation of the isotopic method through an interlaboratory study

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ABSTRACT

L(+)-tartaric acid is one of the most used additives in the oenological practices. Its properties make it useful as preservative and acidulant in grape-derived products. This additive can be obtained through the extraction from grapes, from other fruits or through synthesis from petrochemical sources. In Europe, only L(+)-tartaric acid from grapes can be added to wine products, but the price difference between the forms could lead to the fraudulent use of the cheaper synthetic L(+)-tartaric acid. In other continents where such limitation is not applied, the clear commercial identification of the products is still desirable. Stable isotope analysis of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) proved to be a robust and effective method in discriminating between synthetic and grape-derived L(+)-tartaric acid. In this study, to define the threshold limits of $\delta^{13}C$ (above −24.8 ‰) and $\delta^{18}O$ (above +25.6 ‰) of grape-derived L(+)-tartaric acid, a database consisting of 81 authentic natural samples was created. Moreover, the validation of the isotopic method through an interlaboratory study involving nine European and extra-European laboratories, was provided. The repeatability and the reproducibility standard deviation of the method were calculated.

1. Introduction

L(+)-tartaric acid is a white, crystalline organic acid naturally present in different varieties of fruits, including grapes and tamarinds among others [1,2]. It is widely used as preservative and acidulant in oenological practices, so that the major European wine-producing countries, namely Spain, Germany, Italy, and France, use it more than all United States together [3].

According to the OIV (International Organization of Vine and Wine) monograph (COEI-1-LTARAC) L(+)-tartaric is *"a natural acid extracted from grapes used to acidify musts and wines under conditions stipulated by regulation*". Therefore, in Europe, the OIV prescribes exclusively natural L(+)-tartaric deriving from grape processing waste (lees and pomace) for oenological use. Besides grapes, one of the richest natural sources of L(+)-tartaric acid is tamarind. The extraction process, patented in India and the United States, allows L(+)-tartaric acid to be obtained from an alternative source but which is still commercially too expensive to allow its widespread use in the winemaking field to date [2].

Commercially available L(+)-tartaric acid may derive from both the extraction from vegetal matrices and from synthetic sources such as the fossil oil [4]. The synthetic path involves first the conversion of maleic anhydride, which derives from petroleum byproducts, to an epoxysuccinic acid through the oxidation with H_2O_2, and second the conversion of the epoxysuccinic acid to L(+)-tartaric acid through acidic microbiological hydrolysis [5]. Since the production of the natural form goes through multiple stages, including extraction and crystallisation, it is more expensive than most acidifiers, including citric and malic acids, and much more than synthetic ones [6]. Therefore, the cheaper synthetic form of the oenological adjuvant can be fraudulently (where not permitted by legislation, for example in Europe) used instead of the natural analogue extracted from grapes. Furthermore, the sale of a synthetic analogue passed off as a grape-derived product represents a commercial fraud in any country.

It was therefore necessary to develop new analytical approaches that

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would allow the two forms of acid to be discriminated against each other. The only method currently recognized by the OIV is based on the quantification of carbon 14 (OIV-MA-AS313-23 - *Identification of L-tartaric acid as being of plant or fossil origin by measuring its activity*). Synthetic L(+)-tartaric acid, derived from fossil fuel by-products, has a negligible concentration of carbon 14 compared to the natural grape-derived analog. The ^{14}C concentration is usually expressed as relative amount (%). In fact, in renewable raw materials like plants (0–10 years), ^{14}C activity reaches approximately 100 %, while in higher dated products such as fossil sources, the ^{14}C activity approaches 0 %. Although effective, the method is expensive, time-consuming and only few laboratories in the world are equipped to carry it out.

As demonstrated by Serra et al. and Leirose et al., despite having the same chemical properties, the natural grape-derived and the synthetic forms of L(+)-tartaric acid can still be distinguished based on the stable isotope ratio analysis (SIRA) [4,7].

The measurement of carbon stable isotope ratio ($\delta^{13}C$) alone is not sufficient to differentiate natural L(+)-tartaric acid from the synthetic one, as the petrochemical sources can cover a wide range of $\delta^{13}C$ values, which can overlap with the typical $\delta^{13}C$ of vegetal C3 sources, usually ranging from −30 to −23 ‰ [8]. In this type of plants, carbon enters the photosynthetic cycle as CO_2 exclusively, which is assumed to have a globally constant isotopic composition [9]. Based on published literature data, the $\delta^{13}C$ variability of L(+)-tartaric acid coming from grapes ranges between −23.30 ‰ and −21.11 ‰, while for the analogue deriving from maleic anhydride the same values range between −31.53 ‰ and −20.53 ‰ [4,7]. The SIRA can therefore be used for a preliminary and cost-effective screening to identify synthetic samples with $\delta^{13}C$ values outside the natural range, indicating the synthetic origin of a certain product.

The oxygen atoms inside synthetic L(+)-tartaric acid molecules come from groundwater (in particular from local precipitation) exclusively, which is used in different stages of L(+)-tartaric acid industrial production. On the contrary, the oxygen atoms inside natural L(+)-tartaric acid molecules derive from "vegetative" water, which is a combination of three different sources: precipitation water absorbed through the root system (no ongoing fractionation during this process), atmospheric CO_2 absorbed during photosynthesis and atmospheric O_2, both introduced into the plant through the leaves. The absorption of both CO_2 and O_2 carries oxygen fractionation, due to climatic stress factors such as drought, exposure to light and temperature [10]. Vegetative water and groundwater are known to have different ^{18}O contents, with the latter enriched in heavier isotopes than the former. These significant differences result in products having different oxygen isotopic ratios ($\delta^{18}O$) and allow a clear discrimination between grape-derived and synthetic L(+)-tartaric acid. According to the literature, the $\delta^{18}O$ values of L(+)-tartaric acid coming from grapes range between +23.43 ‰ and +34.20 ‰, while the same values range between +12.99 and + 20.92 ‰ for the synthetic analogue, which can derive, for instance, from maleic anhydride [4,7].

To the best of our knowledge, only a few works reporting the isotopic difference between synthetic and grape-derived L(+)-tartaric acid are available in the literature [4,7]. In both studies the samples were not extracted and purified directly by the authors, but they were rather found on the market in the form of high purity L(+)-tartaric acid. While the difference between the totally synthetic product and the one declared natural from grapes is evident, it is not possible to establish with certainty whether or not some of the natural samples were contaminated with L(+)-tartaric acid of synthetic origin, even though declared by the manufacturer as from grapes. Furthermore, for the $\delta^{18}O$ parameter, either the reference standards used in the mentioned studies were not specified, or a single-point correction was applied, against the *Good practice guide for isotope ratio mass spectrometry* recommendations [11]. The $\delta^{13}C$ and $\delta^{18}O$ were also addressed as a powerful parameter in the geographical discrimination of natural L(+)-tartaric acid, even if a limited number of samples per single geographical origin was considered [7].

In order to define with certainty the cutoff limits for the two isotopic parameters of natural L(+)-tartaric acid in grapes, an in-depth study on a significant number of samples (n = 81) was therefore carried out. To ensure the authenticity of the samples, the L(+)-tartaric acid was directly extracted/converted from calcium tartrate coming from grapes lees and pomace. The results were compared to the $\delta^{13}C$ and $\delta^{18}O$ values of synthetic and commercial samples collected in this project, as well as to isotopic values available in the literature.

This study also aimed to estimate the method validation parameters for both carbon and oxygen isotopic analysis of L(+)-tartaric acid, through a collaborative partnership involving nine European and extra-European laboratories. Five pure L(+)-tartaric acid samples (two synthetic and three natural) were selected and sent in double blind to the other participants joining the interlaboratory study. The samples were analysed through EA-IRMS (Elemental Analyser - Isotope Ratio Mass Spectrometer) for the carbon isotopic ratio analysis and through TC/EA-IRMS (High Temperature Conversion Elemental Analyser - Isotope Ratio Mass Spectrometer) for the oxygen isotopic ratio analysis. Once collected and gathered all data, the repeatability and reproducibility standard deviations of the method were calculated.

2. Material and method

2.1. Participants joining the interlaboratory study

To validate the isotopic method for the measurement of L(+)-tartaric acid $\delta^{13}C$ and $\delta^{18}O$, nine European and extra-European laboratories were considered. Eight of them provided measurements for both $\delta^{13}C$ and $\delta^{18}O$, while one joined the study only for the $\delta^{13}C$ assessment. In agreement with the International harmonised protocol for the proficiency testing of analytical chemistry laboratories, a minimum number of five laboratories, selected according to their competences, was considered [12]. The description of the laboratories which decided to join the partnership is reported in Table 1.

2.2. Samples management

2.2.1. Interlaboratory study samples supply

For the interlaboratory study, Fondazione Mach (hereafter referred to as the Organiser) selected five samples of L(+)-tartaric acid (Tartaric 1–5), including two synthetic products provided on the market and three natural acids produced by Randi Group, according to the protocol described in Session 2.1, and sent them as double blind repetitions (ten samples total) to all the laboratories.

To ensure the reproducibility and repeatability of the analysis in the different laboratories, the Organiser took care of the supply of two International standard materials (IStnd) having $\delta^{13}C$ and $\delta^{18}O$ covering the values variability of L(+)-tartaric acid samples. In particular, standard materials having lower (IStnd-L) and higher (IStnd-H) $\delta^{13}C$ and $\delta^{18}O$ values than L(+)-tartaric acid samples were selected. The description of the standards considered suitable for this purpose is reported in Table 2. Moreover, the Organiser took care to provide instructions about samples and standard preparative, isotopic analysis, batch setup and data correction to all the participants.

2.2.2. Samples description and L(+)-tartaric pilot production

To assess the $\delta^{13}C$ and $\delta^{18}O$ variability of L(+)-tartaric acid from grapes, 81 authentic tartrate samples from five different geographical origins (Australia, Spain, Italy, France and South America, world leader countries for the production of this raw material) have been collected and converted in their acid form. The tartrate was directly taken from lees and pomace inside the barrels, after natural precipitation from the must. For all samples, and particularly for those coming from countries where the use of synthetic L(+)-tartaric as oenological adjuvant is permitted (e.g. Australia), the supplier guaranteed that the samples were

Table 1
Laboratories involved in the interlaboratory study and of the equipment used for the isotopic analysis.

Institution and person in charge	Institution address	Isotope Ratio Mass Spectrometer (IRMS)	Elemental Analyser (EA) + High Temperature Conversion Elemental Analyser (TC/EA)
Fondazione Edmund Mach *Dr. Matteo Perini,* matteo.perini@fmach.it	San Michele all'Adige (Trento), Italy	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific Flash EA 1112 + TC/EA
Agenzia Dogane Monopoli *Dr. Claudia Zedda,* claudia.zedda@adm.gov.it	Laboratorio chimico di Torino (Torino), Italy	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific EA Flash 2000 + TC/EA
Imprint Analytics GmbH *Dr. David Psomiadis,* psomiadis@imprint-analytics.at	Neutal, Austria	Thermo Fisher Scientific Delta V Advantage + HEKAtech GmbH HTO-NU Horizon	Thermo Fisher Scientific Flash EA 1112 + HEKAtech GmbH HTO-NU Horizon
Hydroisotop GmbH - Stable isotope laboratory *Dr. Andrey Voropaev,* AV@Hydroisotop.de	Schweitenkirchen, Germany	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific Flash EA 2000
Agroisolab GmbH *Dr. Claudia Erven,* c.erven@agroisolab.de	Jülich, Germany	HEKAtech GmbH NU Horizon	Eurovector EA + AIL Technologies GmbH High-Temperature Furnace
Eurofins Authenticity Competence Centre *Dr. Freddy Thomas,* freddythomas@eurofins.com	Nantes, France	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific Flash EA 1112 + TC/EA
TLR international laboratories *Dr. Rick Wolf,* rwolf@tlr.nl	Ridderkerk, Nederland *	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific Flash EA 1112
State scientific research Institute of the brewing and wine industry *Dr. Alexander Panasyuk,* alpanasyuk@mail.ru	Moscow, Russia	Thermo Fisher Scientific Delta V Advantage	Thermo Fisher Scientific EA IsoLink CN/OH IRMS
SGS Taiwan Ltd. *Dr. Wan, Naijung,* NJ.WAN@sgs.com	New Taipei City, Taiwan	Thermo Fisher Scientific Delta V Advantage	Uniprep 2-Euro-vector, + Thermo Fisher Scientific EA IsoLink CN/OH IRMS

*TLR international laboratories joined the part of the study regarding the analysis of carbon only.

Table 2
Description, oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic values and reference code of the standards considered in the present interlaboratory study.

Standard description	Certified $\delta^{18}O$ ‰ (vs V-SMOW)	Certified $\delta^{13}C$ ‰ (vs V-PDB)	Reference Code
CBS Caribou Hoof	2.39 ± 0.13	−22.63 ± 0.10*	IStnd-L
USGS90 millet flour from Tuscany, Italy	35.90 ± 0.29	−13.75 ± 0.06	IStnd-H

*The value and the standard deviation of CBS $\delta^{13}C$ were not provided by the USGS, but they were measured in the Organiser laboratory through a ten repetition EA-IRMS analysis.

collected in certified wineries that did not use the acid synthetic form. Together with the natural samples, two other groups of synthetic (8) and commercial (15) L(+)-tartaric were considered. Synthetic and commercial samples were purchased from authorised chemicals suppliers.

To process small quantities of calcium tartrate, the extraction was carried out on a pilot plant, following the same protocol as the one commonly applied by large manufacturing companies. The conversion of tartrate in L(+)-tartaric acid was carried out in the Randi Group laboratory as described below. At the end of the synthesis, L(+)-tartaric acid purity was determined by HPLC (HPLC Agilent Technologies 1260 Infinity II) and polarimetric analysis (Polarimeter Anton Paar MCP150). The HPLC-purity values of the analysed samples resulted above 95 % and the degree measured by polarimetry lies in the specific range of L(+)-tartaric acid (12.0–12.8°). The main procedure steps are represented in Fig. 1 and described as follows.

Step 1, Attack. A stirred water solution of calcium tartrate (1500 g) was added with the stoichiometric amount of sulphuric acid required to dissolve it. Acid additions of about 5 mL avoided excessive foaming due to the possible presence of carbonates in the tartrate. A final sulphuric acid volume of 400–600 mL was necessary to reach the complete dissolution of calcium tartrate. To check the complete dissolution of calcium tartrate (i.e. to consider the attack reaction totally occurred), the excess of sulphates was measured.
Step 2, Filtration. Step 1 solution was filtered on a Büchner filter (110 mm), obtaining a clear solution by separation of the calcium sulphate precipitate.
Step 3–4, First concentration and decantation. The solution of L(+)-tartaric acid underwent a first concentration by heating until values of 50 % w/w. The obtained red solution was decanted to eliminate the whitish and gelling precipitate.
Step 5–6, Second concentration and decantation. The L(+)-tartaric acid solution underwent a second concentration/crystallisation by heating, obtaining a red raw crystal of L(+)-tartaric acid (Fig. 2a).
Step 7, Centrifugation. The red crystal of L(+)-tartaric acid was dried and partially purified by centrifugation using a scaled centrifuge system.
Step 8–9, Redissolving and filtration. The red crystal of L(+)-tartaric acid was redissolved in water, obtaining a solution with a concentration of 50 % w/w, and added with carbon, having a decolorizing function. Then, the solution was filtered over a membrane to eliminate the carbon.
Step 10, Filtration. The clear solution was passed through an ion-exchange resin column to eliminate metal traces.
Step 11, Third concentration. The solution underwent a third concentration/crystallisation by heating, obtaining a white pure L(+)-tartaric acid crystal (Fig. 2b).
Step 12–13, Centrifugation and drying. The L(+)-tartaric acid crystal was dried by centrifugation, using the same scaled centrifuge system as in Step 7, and it was dried at about 60 °C in a laboratory oven.

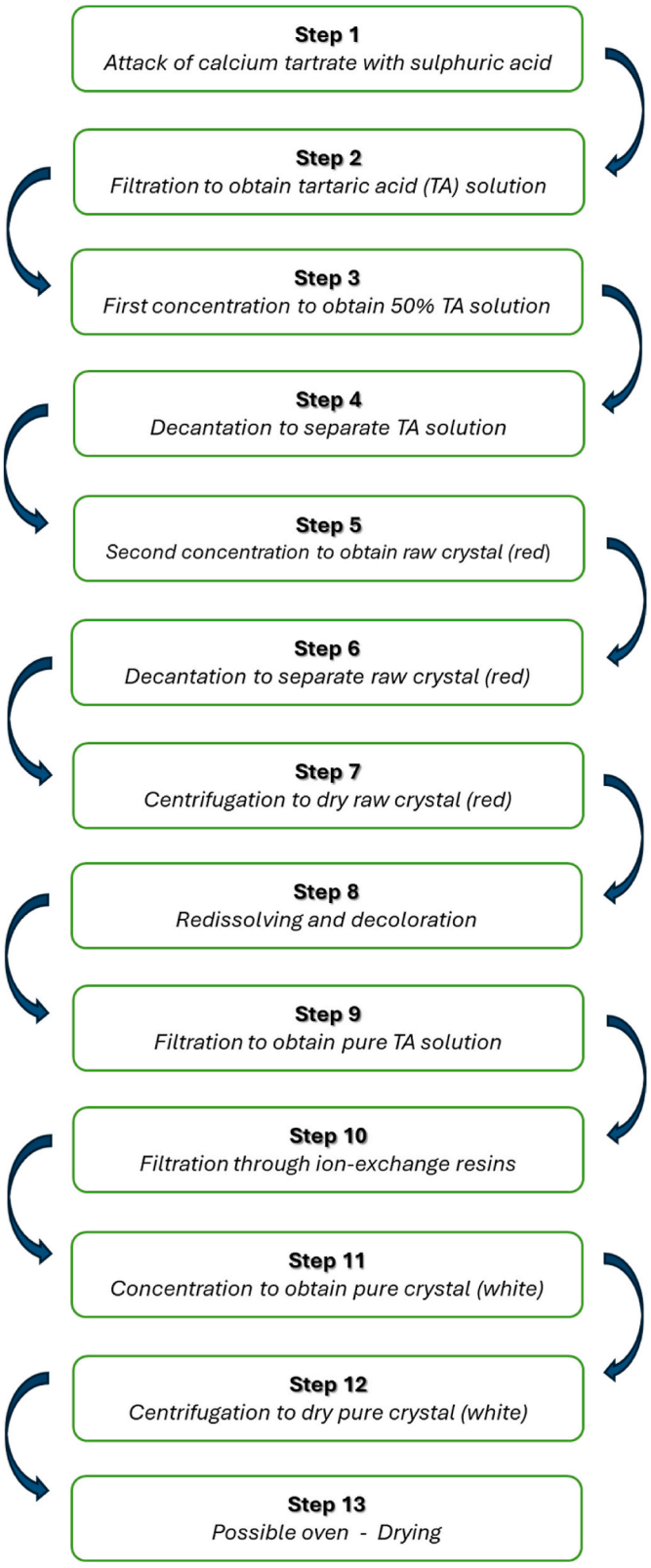

Fig. 1. L(+)-tartaric acid production process carried out in Randi Group laboratory.

2.3. Equipment general description

2.3.1. Elemental Analyser - Isotope Ratio Mass Spectrometer (EA-IRMS)

Although the EA-IRMS brand and settings depend on the laboratory where the analyses were performed, all instruments have similar configurations.

Usually, the instrument is equipped with an oxidation quartz reactor (900–1050 °C), converting all organic matter in CO_2, NO_x and H_2O in an O_2-rich atmosphere, and a reduction quartz reactor (650 °C), removing all the O_2 excess and converting all the NO_x in N_2. The oxidation reactor is usually filled with Cr_2O_3 and Co_3O_4+Ag, while the reduction reactor is generally packed with high purity Cu. Variations depending on the laboratory must be considered [11].

The water is separated through a water trap, while CO_2 and N_2 are separated from each other via gas chromatography. A continuous flow of helium passes through the reactors (helium for analysis, CAS No 07440-59-7, minimum purity 99.999 %). The CO_2 used as reference gas (CAS No 111-72-4) has a minimum purity of 99.998 %.

Table 1 shows the brand and model of the equipment used by the nine laboratories to carry out the $\delta^{13}C$ analysis.

2.3.2. High Temperature Conversion Elemental Analyser - Isotope Ratio Mass Spectrometer (TC/EA-IRMS)

Although the TC/EA-IRMS brand and settings depend on the laboratory where the analyses were performed, all instruments have similar configurations.

Usually, the instrument is equipped with a single reactor converting organic and inorganic compounds in CO, H_2 and N_2 at a high temperature (1350–1450 °C). The reactor consists in an outer tube of fused alumina, containing the inner tube made of glassy carbon and filled with glassy carbon particles and silver wool. Variations depending on the laboratory must be considered [11].

All gases produced from the combustion are separated through gas-chromatography. A continuous flow of helium passes through the reactors (helium for analysis, CAS No 07440-59-7, minimum purity 99.999 %). The CO used as reference gas (CAS No 1641-69-6) has a minimum purity of 99.998 %.

Table 1 shows the brand and model of the equipment used by the nine laboratories to carry out the $\delta^{18}O$ analysis.

2.4. Isotopic analysis protocol applied in the interlaboratory study

Since each laboratory has its own common practices, the Organiser took care to send guidelines for the application of the protocol, with the aim to standardise the procedure as much as possible. The information reported in this session are therefore general indications about how isotopic analyses should have been handled.

The L(+)-tartaric crystals were weighted in tin capsules for carbon and in silver capsules for oxygen isotopic analysis through a micro analytical balance (minimum weight range 0–100 mg and minimum precision 0.01 mg). To calculate the amount of sample to weight, it must be remembered that the difference between the amount of CO_2 (in carbon isotopic analysis) or CO (in oxygen isotopic analysis) produced by the sample and the standards (both IStnd-L and IStnd-H) combustion must not exceed 50 %. The amount of CO_2 and CO produced from all samples are proportional to the intensity of signal 44 *m/z* and 28 *m/z*, respectively. The reference gas flow must be adjusted accordingly to the sample/standard peak intensity.

Samples were analysed at least in duplicate as for $\delta^{13}C$ and in triplicate as for $\delta^{18}O$. All laboratories were suggested to run the international standards provided by the Organiser (IStnd-L and IStnd-H) and internal additional standards (UStnd-L and UStnd-H) at the beginning, in the middle and at the end of the sample batch and then to use them to calculate a two-point calibration curve. Differences between repetitions of the same sample $\leq$0.3 ‰ for the $\delta^{13}C$ and $\leq$0.5 ‰ for the $\delta^{18}O$ were accepted. The obtained corrected results were averaged to give a mean value and expressed with two decimal places.

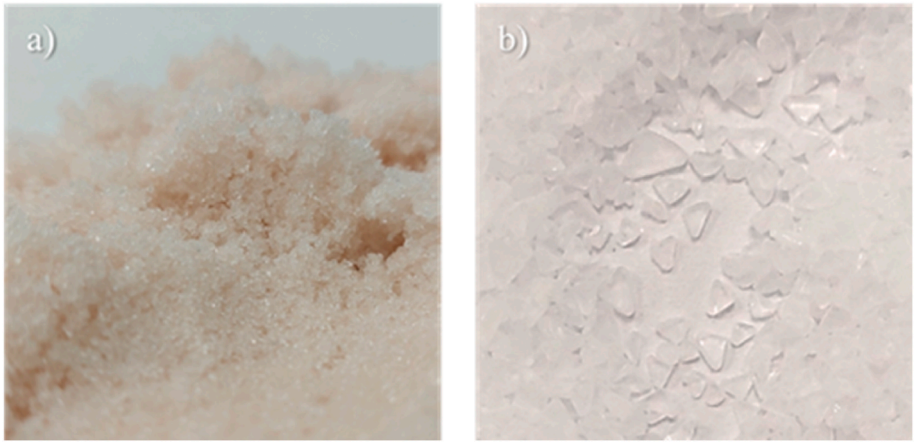

Fig. 2. l(+)-tartaric acid raw wet crystals (red) (a) and pure crystals (white) (b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.5. Isotopic analysis protocol applied in the calculation of grape-derived L(+)-tartaric acid reference values

This activity was conducted entirely in the Organiser's laboratories on the L(+)-tartaric acid samples provided by Randi, in order to define the range of isotopic variability of the product obtained from grape. Isotope ratios were measured using an isotope ratio mass spectrometer (Finnigan DELTA XP, Thermo Scientific, Bremen, Germany) after either total combustion ($\delta^{13}C$) or complete pyrolysis ($\delta^{18}O$) in an elemental analyser (Finningan DELTA TC/EA, high temperature conversion elemental analyser, Thermo Scientific). Approximately 0.8 mg and 0.2 mg of sample were weighted for carbon and oxygen isotopic analyses, respectively. Tin capsules (5 × 9 mm) and silver capsules (5 × 9 mm) were used for carbon and oxygen isotopic analyses, respectively. As for the EA-IRMS working conditions, the oxidation reactor was set to 980 °C, the reduction reactor was set to 650 °C, while for the TC/EA-IRMS the reactor was set at 1410 °C. The ultra-high purity helium continuous flow was 120 mL/min for the EA-IRMS and 90 ml/min for the TC/EA-IRMS, respectively.

For $^{13}C/^{12}C$, the isotopic values were calculated using a two-point calibration curve against working in-house standards (caseins), which were themselves calibrated against international reference materials: fuel oil NBS-22 with $\delta^{13}C = -30.03 \pm 0.05$ ‰, sucrose IAEA–CH–6 with $\delta^{13}C = -10.45 \pm 0.04$ ‰ (IAEA-International Atomic Energy Agency, Vienna, Austria) and L-glutamic acid USGS 40 with $\delta^{13}C = -26.39 \pm 0.04$ ‰ (U.S. Geological Survey, Reston, VA, USA). For $^{18}O/^{16}O$ the isotopic values were corrected using a two-point calibration curve respect to the international certified standards benzoic acid IAEA 601 with $\delta^{18}O = +23.14 \pm 0.19$ ‰ and benzoic acid IAEA 602 with $\delta^{18}O = +71.28 \pm 0.36$ ‰ from IAEA.

2.6. Results management

2.6.1. Results expression

All isotopic ratios were expressed in δ against V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$, and V-SMOW (Vienna-Standard Mean Ocean Water) for $\delta^{18}O$, according to the following equation:

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

where ref is the international measurement standard, sample is the analysed sample and $^{i}E/^{j}E$ is the isotope ratio between heavier and lighter isotope. The delta values are multiplied by 1000 and expressed commonly in units "per mil" (‰) or, according to the International System of Units (SI), in unit 'milliurey' (mUr).

2.6.2. Results correction and calculation

As the raw data usually refer to the standard gases used for the analysis (CO_2 for $\delta^{13}C$ and CO for $\delta^{18}O$), they must be corrected and normalised. A two points correction was applied in both cases, using the international standard with high and low $\delta^{13}C$ and $\delta^{18}O$ (IStnd-L and IStnd-H) provided by the Organiser (see Table 2). The interpolation line equation was finally used to correct sample values.

The internal standards, on the other hand, were used from each laboratory as quality control of the analysis. The mean $\delta^{13}C$ and $\delta^{18}O$ of both UStnd-L and UStnd-H had to fall within the range established by the laboratory itself during the standard calibration.

The data provided by the nine laboratories (five double-blind samples) were rematched by the Organiser using the unique code. As reported in Section 2.4 the two values that have been provided for the $\delta^{13}C$ results from the mean of two replicates each. On the other hand, the $\delta^{18}O$ the two values have been provided results from the mean of three replicates each. The replicates must not differ more than 0.3 ‰ and 0.5 ‰ for carbon and for oxygen respectively.

2.6.3. Statistical analysis

The full $\delta^{13}C$ and $\delta^{18}O$ datasets were analysed using a mixed model approach, treating the laboratory as a random intercept effect. This approach allowed for the simultaneous estimation of the expected shift for all five tartaric acid samples (s_i) and the random variability (σ_l) associated with the different laboratories (l_j).

The general structure of the model was the following:

$$\delta_{ij} = s_i + l_j + \varepsilon_{ij}$$

where.

- δ_{ij} is the measured shift for each laboratory ($\delta^{13}C$ or $\delta^{18}O$)
- s_i is the estimated mean for sample i
- $l_j \sim N(0,\sigma_l^2)$ represents the random effect of the laboratory
- $\varepsilon_{ij} \sim N(0,\sigma^2)$ is the residual randomly distributed error

σ_l was then used to calculate the z-scores of the individual laboratories. The overall quality of the fit was monitored by manually inspecting the residuals diagnostic plots and the caterpillar plot for the random effects. The plots and the R outputs for both models are included in the supplementary information (Table 1S). In general, the models showed a good level of fit in particular for $\delta^{18}O$. The data about $\delta^{13}C$ showed a slightly larger variability.

Statistical analyses were performed in R [12] by using the lme4

package [13]. Expected values and confidence intervals were extracted from the models by using the marginal effects package [14]. The tidyverse ecosystem [15] was used for data handling and visualization. Repeatability (Sr) and reproducibility (SR) standard deviations were calculated according to the ISO 5725-2:2019 and following Reichenbächer et al. [16] for each of the five samples of L(+)-tartaric acid supplied to the laboratories.

3. Results

3.1. Interlaboratory study results

3.1.1. Carbon isotopic ratio: Z-scores and repeatability (Sr) and reproducibility (SR) standard deviation

Data received from the nine laboratories joining the carbon isotopic analyses are reported in Table 3 and are shown in graphical form in Fig. 3. The figure also displays the best estimates for the $\delta^{13}C$ in the five L(+)-tartaric acid samples and their 99 % confidence intervals extracted from the mixed model. They are also summarised in Table 3. The z-scores for the individual laboratories were also calculated from the individual random intercepts and resulted to be: 0.88 for lab1, 0.05 for lab2, 1.04 for lab3, 0.44 for lab4, -0.18 for lab5, -2.37 for lab6, 0.08 for lab7, 0 for lab8 and 0.06 for lab9. All laboratories had z-score values lower than 3 and were considered acceptable and considered in the calculation of the standard deviations. Considering the results provided by the laboratories and adopting a conservative approach by accounting for the worst-case scenario among the five samples analyzed, the accepted standard deviation values for repeatability (Sr) and reproducibility (SR) of the $\delta^{13}C$ parameter are 0.09 ‰ and 0.12 ‰, respectively (Table 3).

3.1.2. Oxygen isotopic ratio: Z-scores and repeatability (Sr) and reproducibility (SR) standard deviation

As for the carbon isotopic analyses, the data relative to oxygen are reported in Table 4 and are shown in graphical form in Fig. 4. The figure also displays the best estimates for the $\delta^{18}O$ in the five L(+)-tartaric acid samples and their 99 confidence intervals extracted from the mixed model. They are also summarised in Table 4. For this element the z-scores for the individual laboratories were the following: 1.29 for lab1, 1.08 for lab2, 0.94 for lab3, -0.98 for lab4, -0.79 for lab5, 0.14 for lab6, -0.73 for lab7 and -0.95 for lab8. All laboratories had absolute z-score values lower than 3 and were considered acceptable and considered in the calculation of the standard deviations. Considering the results provided by the laboratories and adopting a conservative approach by accounting for the worst-case scenario among the five samples analyzed, the accepted standard deviation values for repeatability (Sr) and reproducibility (SR) of the $\delta^{18}O$ parameter are equal to 0.26 ‰ and 1.08 ‰ respectively (Table 4).

Table 3
Results for the carbon isotopic analysis of the collaborative study on the L(+)-tartaric acid. Estimated $\delta^{13}C$ values (‰ vs V-PDB).

sample description	tartaric 1	tartaric 2	tartaric 3	tartaric 4	tartaric 5
number of valid results	9	9	9	9	9
number of replicates	2	2	2	2	2
Estimated $\delta^{13}C$ values (‰ vs V-PDB)	−26.49	−30.21	−23.14	−24.81	−22.71
Upper 99 % CI	−26.41	−30.13	−23.06	−24.73	−22.63
Lower 99 % CI	−26.57	−30.29	−23.22	−24.90	−22.79
Repeatability (Sr)	0.09	0.03	0.03	0.04	0.02
Reproducibility (SR)	0.12	0.12	0.09	0.09	0.09

3.2. Threshold limits for $\delta^{13}C$ and the $\delta^{18}O$ of L(+)-tartaric acid from grape

To establish the isotopic variability of natural L(+)-tartaric acid from grapes, 81 samples with different geographical origin were considered. The results are reported in Table 2S (supplementary) and are summarised in Table 5.

Considering the estimated uncertainty (calculated as 2*standard deviation of reproducibility, see section 3.1), lower threshold values for the $\delta^{13}C$ and the $\delta^{18}O$ were established: L(+)-tartaric acid with $\delta^{13}C$ lower than −24.8 ‰ and $\delta^{18}O$ lower than +25.6 ‰ cannot be considered as 100 % natural from grape. These threshold limits were applied on the commercial samples declared as natural from grape. All samples fell within the defined range of variability and mean values of $\delta^{13}C = -22.6 \pm 0.2$ ‰ and $\delta^{18}O = 29.2 \pm 1.5$ ‰ were calculated for this group (Fig. 5).

The results for the isotopic analyses of both carbon and oxygen are not always consistent with previous studies on L(+)-tartaric acid sources. Serra et al. reported $\delta^{13}C$ ranging from −22.6 ‰ to −21.1 ‰ and $\delta^{18}O$ ranging from 25.2 ‰ to 30.3 ‰ for L(+)-tartaric samples found on the market and declared as natural from grape [4]. Furthermore, in a study on L(+)-tartaric acid produced from both grapes and tamarind, the declared grape-derived product $\delta^{13}C$ varied from −23.9 ‰ to −21.7 ‰ and the $\delta^{18}O$ varied from 23.7 ‰ to 27.7 ‰ [2]. In another study, Leirose et al. reported $\delta^{13}C$ ranging from −23.3 ‰ to −21.8 ‰ and $\delta^{18}O$ ranging from 24.3 ‰ to 34.2 ‰ for the same type of samples [7]. The $\delta^{18}O$ values reported in the literature for samples found on the market (purchased in

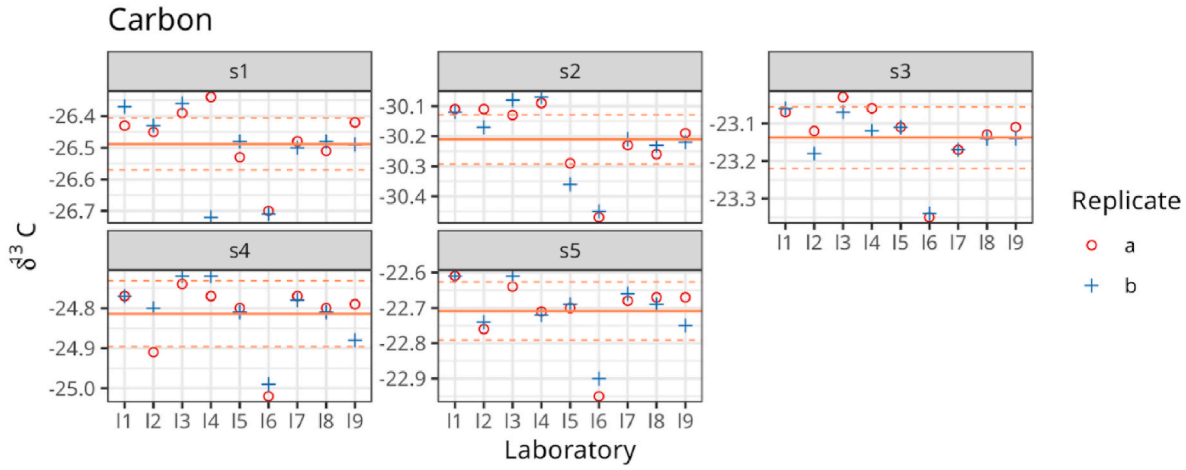

Fig. 3. Carbon isotopic ratios ($\delta^{13}C$) for all the L(+)-tartaric acid samples included in the study. The dots indicate the individual measurements collected at each laboratory (li). The orange line shows the estimated $\delta^{13}C$ for each individual sample. The dashed line shows the 99 % confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

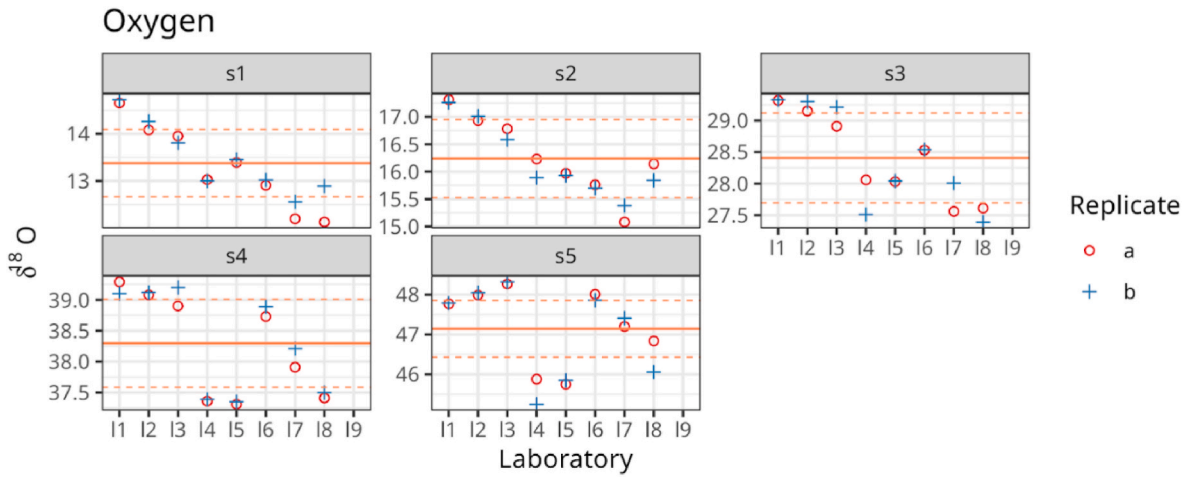

Fig. 4. Oxygen isotopic ratio ($\delta^{18}O$) for all the L(+)-tartaric acid samples included in the study. The dots indicate the individual measurements collected at each laboratory (li). The orange line shows the estimated $\delta^{18}O$ for each individual sample. The dashed line shows the 99 % confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Results for the carbon isotopic analysis of the collaborative study on the L(+)-tartaric acid. Estimated $\delta^{18}O$ values (‰ vs V-PDB).

sample description	tartaric 1	tartaric 2	tartaric 3	tartaric 4	tartaric 5
number of valid results	8	8	8	8	8
number of replicates	2	2	2	2	2
Estimated $\delta^{18}O$ values (‰ vs V-PDB)	13.38	16.24	28.41	38.30	47.14
Upper 99 % CI	14.09	16.95	29.12	39.01	47.86
Lower 99 % CI	12.67	15.52	27.69	37.58	46.43
Repeatability (Sr)	0.22	0.15	0.20	0.13	0.26
Reproducibility (SR)	0.83	0.69	0.74	0.84	1.08

Table 5
Carbon and oxygen isotopic variability for natural L(+)-tartaric acid; the isotopic values of both carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) are reported together with the standard deviation and the calculated threshold limit below which the sample can not be considered 100 % natural from grape.

	$\delta^{13}C$ (‰, vs V-PDB)	$\delta^{18}O$ (‰, vs V-SMOW)
Mean	−23.3	+34.3
Standard deviation	0.6	7.4
Lower limits	−24.6*	+27.8**

*To establish the threshold value for the $\delta^{13}C$, an uncertainty of 0.2 ‰ must be considered.
**To establish the threshold value for the $\delta^{18}O$, an uncertainty of 2.2 ‰ must be considered.

their L(+)-tartaric acid form) and below the estimated limit (e.g. +23.7 ‰) could be explained by the addition of relatively low amounts of the synthetic analogue to a natural product, resulting in lower $\delta^{18}O$ values.

The $\delta^{18}O$ of water is strongly representative of a specific geographical location, as it depends on parameters such as latitude, altitude and closeness to the sea [17]. Therefore, a relationship between the sampling site and the $\delta^{18}O$ of the L(+)-tartaric acid produced in that area was expected.

Moreno Rojas et al. reported that L(+)-tartaric acid samples from Spain have higher $\delta^{18}O$ values than French and Italian ones [2]. Leirose et al. reported significantly lower values in samples from South American L(+)-tartaric acid (average $\delta^{18}O = +24.79$ ‰) compared to the European ones (average $\delta^{18}O = +29.25$ ‰) [7]. However, this correspondence could be distorted by the addition of synthetic L(+)-tartaric acid to a natural product.

The tartrate samples considered in our study came from different geographical areas (Australia, France, Spain, Italy and South America). The $\delta^{18}O$ values found in the extracted L(+)-tartaric acid did not show significant differences depending on the different origins. This evidence must be further investigated with specific studies.

The mean values of the synthetic samples considered in this study were $\delta^{13}C = -27.8 \pm 3.2$ ‰ and $\delta^{18}O = 15.3 \pm 1.9$ ‰. Synthetic samples presented $\delta^{13}C$ and $\delta^{18}O$ values statistically lower than the natural grape-derived ones (T-Test, $p < 0.05$). The results are in agreement with previous studies involving synthetic L(+)-tartaric acid samples, reporting mean $\delta^{13}C$ and $\delta^{18}O$ values of −25.1 ‰ and 17.4 ‰, respectively [7]. Anyways, the aim of this study was not to define a limit for the synthetic product, but rather to further support the evidence already reported by other authors regarding the significant differences between synthetic and grape-derived L(+)-tartaric acid isotopic values.

3.3. *Evaluation of possible sources of isotopic variability*

The L(+)-tartaric acid considered in this work to study both carbon and oxygen isotopic composition was obtained in the laboratory according to Fig. 1. Since the scheme includes several physical and chemical processes, their possible effect on both $\delta^{13}C$ and $\delta^{18}O$ isotopic ratios was evaluated. Both carbon and oxygen isotopic fractionation can be excluded in steps such as the use of activated carbon, the use of the ion exchange resin and the drying process, since during the conversion of tartrate into L(+)-tartaric acid, the former does not change its structure. Therefore, the separation of the individual atoms (carbon, hydrogen and oxygen) and subsequent recombination with reformation of the ion does not occur.

On the other hand, the entire conversion process of tartrate into L(+)-tartaric acid takes place in aqueous solution, so water $\delta^{18}O$ value could affect the L(+)-tartaric acid one due to a possible equilibrium fractionation. To exclude this eventuality, we considered the same tartrate and we converted it into L(+)-tartaric acid using two isotopically different water samples ($\delta^{18}O = -8.5$ ‰ and $\delta^{18}O = +2.5$ ‰, respectively). The $\delta^{18}O$ of the two resulting L(+)-tartaric acid samples showed no statistically significant differences, considering the estimated analytical uncertainty (see section 3.1).

The sample size, the different geographical origins considered and the use of an extraction process identical to the industrially used one contribute to the robustness of the method provided in this study.

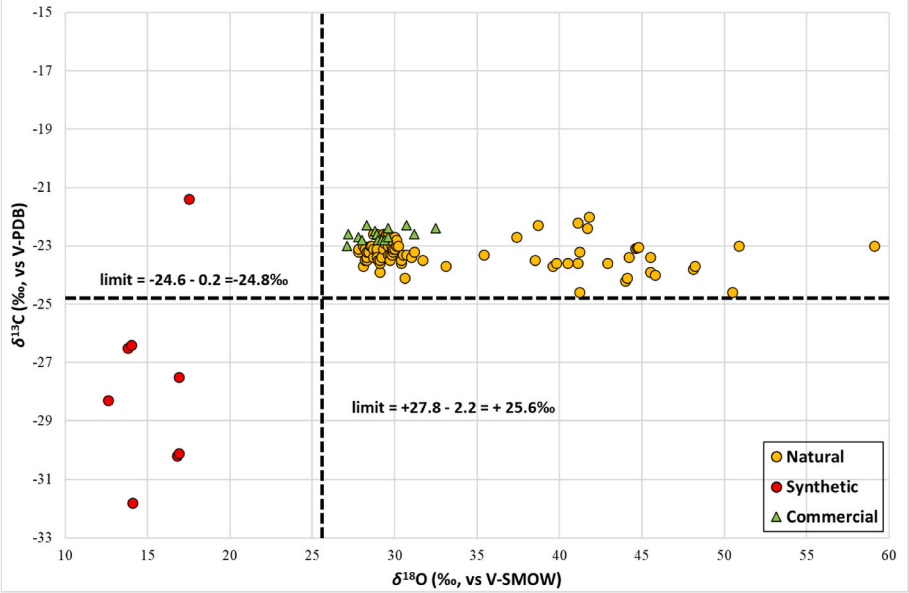

Fig. 5. Correlation between carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopic ratios of natural, synthetic and commercial (declared as grape-derived) L(+)-tartaric acid samples.

4. Conclusion

For the first time, threshold values of $\delta^{13}C$ and $\delta^{18}O$ of grape-derived L(+)-tartaric acid were provided through a robust study based on a wide database and on the geographical representativeness of the samples. Eighty-one tartrate samples from sites located in various wine-producing countries around the world were directly extracted from grapes lees and pomace and converted into L(+)-tartaric acid. The isotopic analysis of the natural samples obtained through this procedure, resembling the industrially used one, made it possible to set threshold values for both $\delta^{13}C$ and $\delta^{18}O$. Indeed, commercial samples reporting $\delta^{13}C$ values below −24.8 ‰ and $\delta^{18}O$ below +25.6 ‰ cannot be considered 100 % natural from grapes.

Thanks to an interlaboratory study, involving nine laboratories of different nationalities, which provided results with Z-score<3 as for all samples and parameters, the standard deviations of repeatability and reproducibility were set for $\delta^{13}C$ (0.09 ‰ and 0.12 ‰, respectively) and $\delta^{18}O$ (0.26 ‰ and 1.08 ‰, respectively) parameters.

CRediT authorship contribution statement

Matteo Perini: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Silvia Pianezze:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Pienti:** Formal analysis. **Giovanna Randi:** Resources, Conceptualization. **Pietro Franceschi:** Software. **Roberto Larcher:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2025.128071.

Data availability

Data will be made available on request.

References

[1] C.A. Batt, R.K. Robinson, Encyclopedia of Food Microbiology, second ed., Academic Press, London, 2014.
[2] J.M. Moreno Rojas, S. Cosofret, F. Reniero, C. Guillou, F. Serra, Control of oenological products: discrimination between different botanical sources of L-tartaric acid by isotope ratio mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 2447–2450, https://doi.org/10.1002/rcm.3105.
[3] B. Caballero, P. Finglas, F. Toldra, Encyclopedia of Food Sciences and Nutrition, second ed., Elsevier Science, Amsterdam, 2003.
[4] F. Serra, F. Reniero, C.G. Guillou, J.M. Moreno, J.M. Marinas, F. Vanhaecke, ^{13}C and ^{18}O isotopic analysis to determine the origin of L-tartaric acid, Rapid Commun. Mass Spectrom. 19 (2005) 1227–1230, https://doi.org/10.1002/rcm.1918.
[5] J.M. Church, R. Blumberg, Synthesis of tartaric acid, by the hydroxylation of maleic acid, Ind. Eng. Chem. 43 (1951) 1780–1786.
[6] J.B. Gurtler, T.L. Mai, PRESERVATIVES | traditional preservatives – organic acids, in: Encyclopedia of Food Microbiology, second ed., Elsevier, Amsterdam, 2014, pp. 119–130, https://doi.org/10.1016/B978-0-12-384730-0.00260-3.
[7] G.D. Leirose, M. Grenier-loustalot, A.H. Oliveira, Investigation of geographical origin and production method of L(+)-tartaric acid by isotopic analyses with chemometrics, J. Chem. Chem. Eng. 11 (2017), https://doi.org/10.17265/1934-7375/2017.02.001.
[8] M.H. O'Leary, Carbon Isotopes in Photosynthesis: fractionation techniques may reveal new aspects of carbon dynamics in plants, Bioscience 38 (1988) 328–336, https://doi.org/10.2307/1310735.
[9] G.D. Farquhar, M.H. O'Leary, J.A. Berry, On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, Funct. Plant Biol. 9 (1982) 121–137, https://doi.org/10.1071/PP9820121.
[10] M.M. Barbour, Stable oxygen isotope composition of plant tissue: a review, Funct. Plant Biol. 34 (2007) 83–94, https://doi.org/10.1071/FP06228.
[11] P.J.H. Dunn, J.F. Carter, Publication of the second edition of the FIRMS good practice guide for isotope ratio mass spectrometry, Isot. Environ. Health Stud. 54 (2018) 656–657, https://doi.org/10.1080/10256016.2018.1531495.
[12] Website, (n.d.). R Core Team (2025). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
[13] D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models Usinglme4, J. Stat. Software 67 (2015), https://doi.org/10.18637/jss.v067.i01.

[14] V. Arel-Bundock, N. Greifer, A. Heiss, How to interpret statistical models using marginaleffects for R and Python, J. Stat. Software 111 (2024), https://doi.org/10.18637/jss.v111.i09.
[15] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the tidyverse, J. Open Source Softw. 4 (2019) 1686.
[16] M. Reichenbächer, J.W. Einax. Interlaboratory Studies Challenges in Analytical Quality Assurance, Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, pp. 305–317.
[17] E. Galimov, The Biological Fractionation of Isotopes, first ed., Elsevier, Amsterdam, 2012.